

Open Research Online

The Open University's repository of research publications
and other research outputs

Isolation, characterisation and some synthesis studies on insecticidal natural products from Sri Lankan plants

Thesis

How to cite:

Hemalal, Kirihehttiliyana Patrick (1998). Isolation, characterisation and some synthesis studies on insecticidal natural products from Sri Lankan plants. PhD thesis. The Open University.

For guidance on citations see [FAQs](#).

© 1998 Kirihehttiliyana Patrick Hemalal

Version: Version of Record

Copyright and Moral Rights for the articles on this site are retained by the individual authors and/or other copyright owners. For more information on Open Research Online's data [policy](#) on reuse of materials please consult the policies page.

oro.open.ac.uk

**ISOLATION, CHARACTERISATION AND
SOME SYNTHESIS STUDIES
ON INSECTICIDAL NATURAL PRODUCTS
FROM SRI LANKAN PLANTS**

by

KIRI HETTILİYANAGE

~~K~~ Patrick Hemalal, M. Phil.

A thesis presented in partial fulfilment of the
requirements for the degree of Doctor of Philosophy of the
Open University
November, 1998

Date of award: 9 February 1999

*Department of Biological and Ecological Chemistry
IACR-Rothamsted
Harpenden Herts
AL5 2JQ
UK*

ProQuest Number: 27696801

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 27696801

Published by ProQuest LLC (2019). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

ABSTRACT

Four Sri Lankan plant species, *Acronychia pedunculata* (L.) Miq (Rutaceae), *Pleurostyliia opposita* (Wall) Alston (Celastraceae), *Alseodaphne semicarpifolia* (Lauraceae) and *Walsura piscidia* Roxb. (Meliaceae) were investigated for insecticidal activity.

Acrovestone (1), was identified as the major insecticidal principle from *A. pedunculata*. New reactions conditions were developed for its synthesis from isovaleraldehyde and two key intermediates, acronylin and demethylacronylin. The latter intermediate was synthesised by optimisation of a previously reported method. This is the first report of the synthesis of acrovestone and recognition of its insecticidal activity.

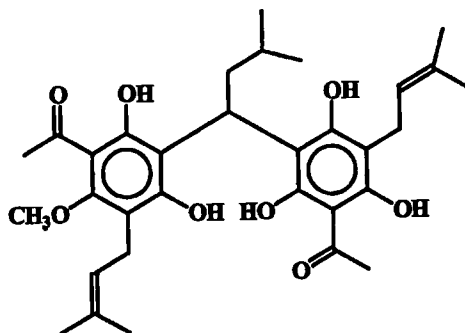
A structure-activity relationship programme, based on synthetic analogues incorporating small but systematic structural changes, revealed that the number, position and size of substituents were all critical for activity. Optimum activity was associated with the presence of a single methoxy group and that only the central region was amenable to variation leading to increased activity. Recognition of the structural similarities between acrovestone and another class of insecticidal products, the kunzeins, prompted an extension of the SAR study. A series of novel 'hybrid' compounds incorporating structural features from both classes was synthesised and tested. In contrast to the conclusions from the systematic SAR study, this part of the study indicated that substantial variations could be tolerated in one segment of the acrovestone molecule leading to analogues with higher levels of

activity than the individual parent compounds. Overall this study has demonstrated the advantage of combining a conventional SAR approach based on small but systematic structural changes with one using larger changes through synthesis of hybrid compounds from related natural products. Limited evaluation against other species indicated variation in the spectrum of activity with chemical structure.

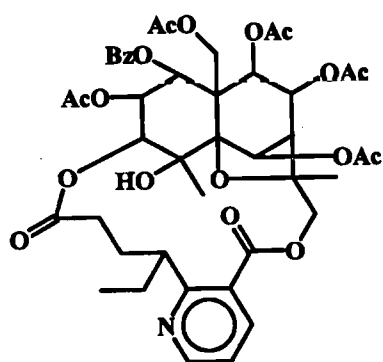
Previously reported insecticidal compounds of *P. opposita* were re-isolated and structures reassigned by means of chemical degradation and spectroscopic methods. They were identified as sesquiterpene polyol ester alkaloids, compounds 2, 3 and 4 containing a previously unknown combination of 6,6 β -dihydroagarofuran core with an alkaloidal fragment.

Sesamin (5) was isolated as the insecticidal component from *A. semicarpifolia*. This is the first report of its isolation from the genus *Alseodaphne*.

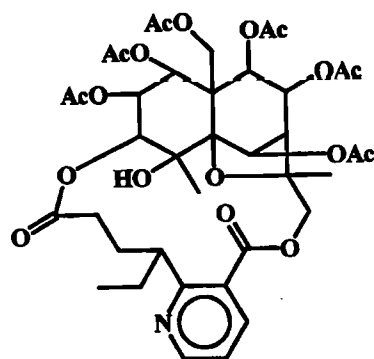
7-Deacetyl-7 α -hydroxyazadirone (6) was isolated as the insecticidal component from *W. piscidia*. This is the first report of its insecticidal activity and its isolation from the genus *Walsura*.



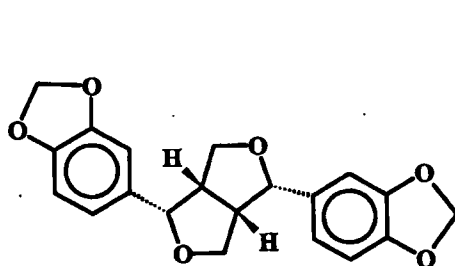
1- *Acronychia pedunculata*



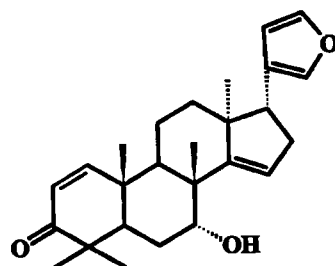
2- *Pleurostylia opposita*



3- *Pleurostylia opposita*



5- *Alseodaphne semicarpifolia*



6- *Walsura piscidia*

Figure 1 : Insecticidal natural products from Sri Lankan plants

ACKNOWLEDGEMENTS

I would like to express my deep gratitude to my supervisor Dr B P S Khambay of the Department of Biological and Ecological Chemistry, IACR-Rothamsted for his continuous supervision, guidance, support and encouragement over the past four years. I am grateful to Dr D Batty for his assistance and day to day supervision in my first two years at Rothamsted.

I gratefully acknowledge Drs Mark Luszniak, Diane Luszniak, N F Janes and Professor Savithri Kumar for their help in editorial work of this manuscript. I am also very grateful to Professor J Pickett, Head of Department, Biological and Ecological Chemistry at Rothamsted for his guidance and encouragement.

My sincere thanks go to Dr Mike Birkett and Sangita Manior for providing Mass spectral data, Mark Hedges, Philip Jewess and Professor Monique Simmonds for bioassay data. I acknowledge the British Technology Group plc and BBSRC for funding this work. I also acknowledge the University of Colombo, Sri Lanka for supplying plant material.

My deep appreciation go to my mother, father, brother and sister for their support throughout this study. I must also say a special word of thank to Dr R Jayamaha for her moral support and continuous help during my Ph.D. studies. Finally, my deepest appreciation and thanks go to my wife Radhika for her encouragement and moral support throughout this study.

TABLE OF CONTENTS

| | |
|-------------------|-----|
| Title | i |
| Abstract | ii |
| Acknowledgements | v |
| Table of Contents | vi |
| Abbreviations | xii |

CHAPTER 1

INTRODUCTION

| | | |
|-------|----------------------------------|----|
| 1.1 | INTRODUCTION | 2 |
| 1.2 | OBJECTIVES OF THE THESIS | 2 |
| 1.3 | BACKGROUND | 2 |
| 1.4 | NATURAL PRODUCTS AS INSECTICIDES | 7 |
| 1.4.1 | Introduction | 7 |
| 1.4.2 | Pyrethrins | 8 |
| 1.4.3 | Nicotine and neonicotinoids | 17 |
| 1.4.4 | Carbamates | 19 |
| 1.4.5 | Rotenoids (Isoflavonoids) | 22 |
| 1.4.6 | Ryania | 23 |
| 1.4.7 | Veratrum Alkaloids | 24 |
| 1.4.8 | Neem | 27 |
| 1.4.9 | Toosendanin | 28 |

| | | |
|--------|--|----|
| 1.4.10 | Isobutylamides | 29 |
| 1.4.11 | Benzofurans | 31 |
| 1.4.12 | Coumarins | 32 |
| 1.4.13 | Lignans | 32 |
| 1.4.14 | Acetogenins | 33 |
| 1.4.15 | Limonoids | 34 |
| 1.4.16 | Sesquiterpenoids | 35 |
| 1.4.17 | Naphthoquinones | 36 |
| 1.4.18 | Insecticides of microbial origin | 37 |
| 1.5 | ASSAYS FOR DETECTION OF INSECTICIDAL NATURAL PRODUCTS | 44 |

CHAPTER 2

RESULTS AND DISCUSSION

| | | |
|-------|--|----|
| 2.1 | INTRODUCTION | 46 |
| 2.2 | ISOLATION AND CHARACTERISATION OF INSECTICIDAL COMPOUNDS FROM <i>ACRONYCHIA PEDUNCULATA</i> | 48 |
| 2.2.1 | Introduction | 48 |
| 2.2.2 | Isolation of insecticidal compounds | 50 |
| 2.2.3 | Characterisation of compound 1 | 51 |

| | | |
|-------|---|----|
| 2.3 | ISOLATION AND CHARACTERISATION OF INSECTICIDAL COMPOUNDS FROM <i>PLEUROSTYLIA OPPOSITA</i> | 56 |
| 2.3.1 | Introduction | 56 |
| 2.3.2 | Isolation of insecticidal compounds | 59 |
| 2.3.3 | Spectroscopic and structural studies of compound 2 | 59 |
| 2.3.4 | Characterisation of compound 3 | 68 |
| 2.3.5 | Characterisation of compound 4 | 70 |
| 2.4 | ISOLATION AND CHARACTERISATION OF INSECTICIDAL COMPOUNDS FROM <i>ALSEODAPHNE SEMICARPIFOLIA</i> | 73 |
| 2.4.1 | Introduction | 73 |
| 2.4.2 | Isolation of insecticidal compounds | 74 |
| 2.4.3 | Characterisation of compound 5 | 74 |
| 2.5 | ISOLATION AND CHARACTERISATION OF INSECTICIDAL COMPOUNDS FROM <i>WALSURA PISCIDIA</i> | 78 |
| 2.5.1 | Introduction | 78 |
| 2.5.2 | Isolation of insecticidal compound | 79 |
| 2.5.3 | Characterisation of compound 6 | 80 |
| 2.6 | INSECTICIDAL ACTIVITY | 82 |
| 2.7 | SYNTHESIS STUDIES OF ACROVESTONE AND ANALOGUES | 85 |
| 2.7.1 | Introduction | 85 |
| 2.7.2 | The postulated biosynthesis of acrovestone | 85 |
| 2.7.3 | Synthesis of acrovestone | 87 |

| | | |
|--------|---|-----|
| 2.7.4 | Synthesis of demethylacronylin | 90 |
| 2.7.5 | Synthesis of acronylin | 95 |
| 2.7.6 | Regioselective synthesis of acronylin | 97 |
| 2.7.7 | Synthesis of acronylin analogues | 100 |
| 2.7.8 | Model reactions for the condensation of polyhydroxy phenols | 105 |
| 2.7.9 | Synthesis of acrovestone and demethylacrovestone | 110 |
| 2.7.10 | Semisynthetic derivatives of acrovestone | 112 |
| 2.7.11 | Synthesis of methoxy analogues of acrovestone | 115 |
| 2.7.12 | Synthesis of acrovestone analogues | 118 |
| 2.7.13 | Synthesis of demethylacrovestone analogues | 120 |
| 2.8 | SYNTHESIS OF ACROVESTONE-KUNZEIN HYBRID MOLECULES | 122 |
| 2.8.1 | Introduction | 122 |
| 2.8.2 | Synthesis of acro-kunzein hybrid compounds, 2.103 and 2.104 | 125 |
| 2.8.3 | Synthesis of acro-kunzein hybrid compounds, 2.105 and 2.106 | 130 |
| 2.9 | SYNTHESIS OF CONSTRAINED-ACROVESTONE ANALOGUES | 130 |
| 2.9.1 | Introduction | 130 |
| 2.9.2 | Synthesis of prenylated derivatives of robustadiols | 131 |
| 2.10 | STRUCTURE -ACTIVITY RELATIONSHIPS STUDIES OF ACROVESTONE | 137 |
| 2.10.1 | Introduction | 137 |

| | | |
|--------|--|-----|
| 2.10.2 | Structural variations and insecticidal activity of acrovestone | 137 |
| 2.10.3 | Hybrid compounds | 146 |
| 2.11 | MODE OF ACTION OF ACROVESTONE | 149 |
| 2.12 | OTHER BIOLOGICAL ACTIVITIES OF ACROVESTONE AND ANALOGUES | 149 |
| 2.13 | OVERALL CONCLUSION | 154 |

CHAPTER 3

EXPERIMENTAL

| | | |
|--------|--|-----|
| 3.1 | GENERAL EXPERIMENTAL | 156 |
| 3.1.1 | Collection and preparation of plant material | 156 |
| 3.1.2 | Extraction | 156 |
| 3.1.3 | Chromatography | 157 |
| 3.1.4 | Spectroscopy | 158 |
| 3.1.5 | Purification of chemicals | 160 |
| 3.1.6 | Insecticidal bioassay | 160 |
| 3.1.7 | Brine shrimp lethality bioassay | 161 |
| 3.1.8 | Fungicidal bioassay | 162 |
| 3.1.9 | Antibacterial bioassay | 162 |
| 3.1.10 | Mode of action studies | 162 |

| | | |
|-------|--|-----|
| 3.2 | EXTRACTION AND ISOLATION OF INSECTICIDAL COMPOUNDS FROM <i>ACRONYCHIA PEDUNCULATA</i> | 163 |
| 3.2.1 | Isolation of compound 1 | 163 |
| 3.3 | EXTRACTION, ISOLATION AND STRUCTURAL STUDIES OF COMPOUNDS FROM <i>PLEUROSTYLIA OPPOSITA</i> | 164 |
| 3.3.1 | Isolation of compounds 2, 3 and 4 | 164 |
| 3.3.2 | Structural studies of compound 2 | 170 |
| 3.4 | EXTRACTION AND ISOLATION OF INSECTICIDAL COMPOUNDS FROM <i>ALSEODAPHNE SEMICARPIFOILA</i> | 171 |
| 3.4.1 | Isolation of compounds 5 | 171 |
| 3.5 | EXTRACTION AND ISOLATION OF INSECTICIDAL COMPOUNDS FROM <i>WALSURA PISCIDIA</i> | 172 |
| 3.5.1 | Isolation of compound 6 | 172 |
| 3.6 | SYNTHESIS PROCEDURES | 174 |
| | REFERENCES | 270 |
| | APPENDIX 1 - Bioassay procedures | |
| | APPENDIX 2 - ^1H and ^{13}C NMR spectra of compounds 1 to 6 | |

ABBREVIATIONS

| | |
|--------------------------------------|---|
| AcCl | Acetyl chloride |
| AlCl ₃ | Aluminium chloride |
| API | Atmospheric pressure ionisation |
| AgNO ₃ | Silver nitrate |
| Bz | Benzoyl |
| br | Broad |
| BF ₃ -Et ₂ O | Borontrifluoride-etherate |
| ¹³ C- ¹ H COSY | Carbon-Hydrogen Correlation Spectroscopy |
| CuI | Copper iodide |
| DEAD | Diethyl azodicarboxylate |
| DEPT | Distortionless enhancement by polarisation transfer |
| DDT | Dichloro diphenyl trichloroethane |
| DMF | N,N-Dimethylformamide |
| DMSO | Dimethyl sulphoxide |
| Et ₃ N | Triethylamine |
| ¹ H- ¹ H COSY | Hydrogen-hydrogen correlation spectroscopy |
| HPLC | High performance liquid chromatography |
| IR | Infra red |
| KOH | Potassium hydroxide |
| LiOMe | Lithium methoxide |
| Me | Methyl |

| | |
|-------------------------|---|
| MOMCl | Methoxy methyl chloride |
| NaOMe | Sodium methoxide |
| Nic | Nicotinyl |
| NADH | Nicotinamide Adenine Dinucleotide, reduced form |
| NIS | N-iodosuccinimide |
| NMR | Nuclear magnetic resonance |
| NOESY | Nuclear Overhauser Enhancement (and Exchange) Spectroscopy |
| PPh₃ | Triphenyl phosphine |
| p TSA | Para-toluenesulphonic acid |
| PTLC | Preparative thin layer chromatography |
| SAR | Structure-activity relationship |
| tlc | Thin layer chromatography |
| TFA | Trifluoroacetic acid |
| TFAA | Trifluoroacetic anhydride |
| TiCl₄ | Titanium tetrachloride |
| TMS | Tetramethylsilane |
| THF | Tetrahydrofuran |
| UV | Ultraviolet |
| ZnCl₂ | Zinc chloride |

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Insecticides play a vital role in modern integrated pest management programmes. The demand for novel classes of insecticides with new modes of action remains unchanged due to the perpetual development of insect resistance against existing insecticides. Plants are recognised as a good source of both natural insecticides and providing lead structures for the development of synthetic analogues. The work embodied in this thesis involves the investigation of insecticidal natural products of plants collected from Sri Lanka.

1.2 OBJECTIVES OF THE THESIS

The objectives of this study are three fold. The first is the separation, identification and determination of insecticidal natural products from plants. The second is to confirm their structures by total synthesis. The third and perhaps the most important objective is to establish structure activity relationships (SAR) of insecticidal compound/s with the aim of identifying structural features that are necessary for insecticidal activity.

1.3 BACKGROUND

The struggle between man and pest became a serious problem at the beginning of the farming age. With the increasing human population and limited food resources it was necessary to expand arable land size, which in turn resulted in

major changes to the ecosystem. Since more and more man-made ecosystems have been established many fauna have invaded the systems as pests. The crops were damaged by pests and plant diseases were introduced. As a result, to date, one third of the world's food production is destroyed annually by pests. The different types of crop losses are indicated in Figure 1.1¹.

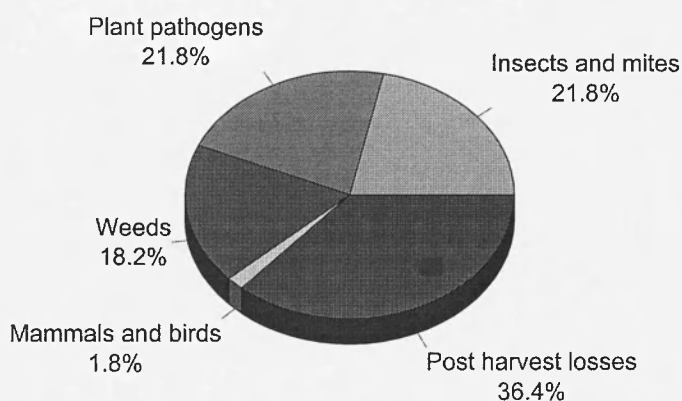


Figure 1.1 : World crop losses (1996)

In the early days cultivational controls such as rotational farming, selection of resistant crops and physical controls and later, inorganic substances were used for crop protection². Gradually different chemicals with pest control properties were used in man-made ecosystems. Later, these chemicals were categorised as herbicides, fungicides, insecticides *etc.*

Use of these chemicals has become a necessary practice for the supply of quality food. The use of agrochemicals in the world today is illustrated in

Figure 1.2³, which shows that insecticides represent the second biggest type of agrochemical used in agriculture today.

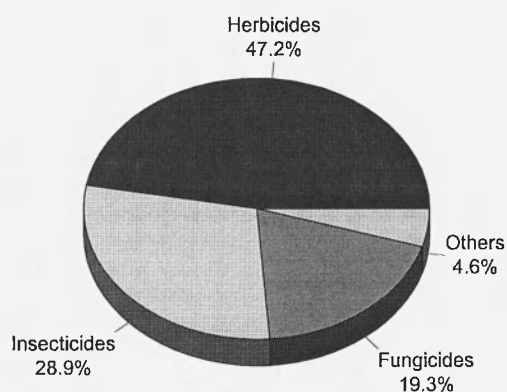


Figure 1.2 : World agrochemical usage (1998)

The origin of insecticides is lost in antiquity, but centuries ago, the Chinese used pyrethrum and derris species and the Romans used hellebore species as insect control agents^{4, 5}. In early days, spices, such as cinnamon, mustard, nutmeg and pepper were used to protect food from insects⁵. About one hundred years ago inorganic materials including white wash, copper and sulphur were also being used extensively for pest control⁶.

In the early twentieth century, botanical insecticides, such as pyrethrum, nicotine and rotenone held prominent places among pest control products. The discovery of synthetic insecticides particularly the organochlorines, organophosphates, carbamates, and recently, the pyrethroids relegated the use of botanicals to secondary markets, such as home, garden and veterinary uses. These synthetic insecticides were discovered primarily by carrying out trial

and error structural changes to lead molecules with the aim of improving insecticidal properties and lowering mammalian toxicity. More recently, the use of computer-assisted quantitative structure activity relationship (QSAR) approaches have provided increasingly useful results in developing new insecticides.

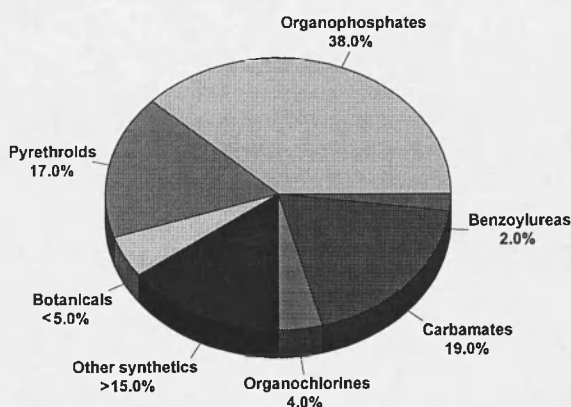


Figure 1.3 : World insecticide usage (\$m) -1996

The main classes of insecticides currently used in the agrochemical market⁷ are indicated in Figure 1.3. Long term use of insecticides has had some drawbacks. Some of the earlier synthetic pesticides especially, the organo chlorines (e.g. DDT, lindane, Figure 1.4) were environmentally non-friendly having long persistence and this resulted in bio accumulation through the food chains⁸. The most important as well as the most worrying drawback is the development of resistance by key pests to the current major classes of synthetic insecticides⁹. The development of new pesticides, especially those with novel modes of action therefore continues to be a major priority for the agrochemical industry.

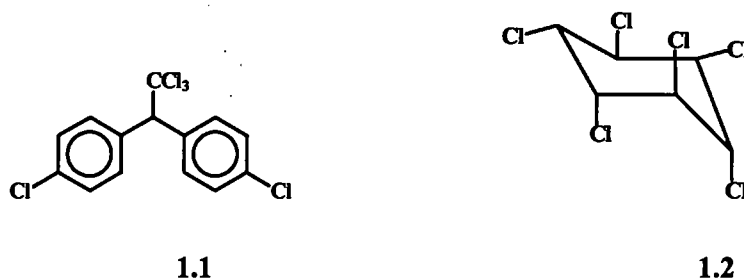


Figure 1.4 : DDT and Lindane

In the course of finding new classes of chemicals for insect control, synthetic programmes are helpful but it is becoming increasingly difficult to discover new lead structures solely by this means. On the other hand, natural products are well known to provide a vast range of bio-active compounds which can be exploited either as leads for further chemical synthesis or for direct use. Currently, five botanical insecticides, pyrethrum, rotenone, ryania, sabadilla and neem are registered for use in the United State (Table 1.1)¹⁰.

Table 1.1 : Current botanical insecticides

| Plant | Country of Origin | Active Ingredients | % TGAC ^a | US\$Kg ^b |
|-----------|-------------------|-------------------------|---------------------|---------------------|
| Pyrethrum | Persia | Pyrethrins, Cinerins | 25.0 | 75 |
| Derris | Peru | Rotenoids (isoflavones) | 7.3 | 3 |
| Ryania | Trinidad | Ryanodine alkaloids | 0.1 | 3 |
| Sabadilla | Venezuela | Veratrum alkaloids | 0.8 | 8 |
| Neem | India | Tetranortriterpenoids | 25.0 | 37.5 |

^aTechnical grade active concentrations. ^b Approximate sale price in 1992

The plant kingdom is known to comprise approximately 500 000 different species¹¹. A vast range of secondary metabolites having different biological activities are produced by these plants. To date, investigation is limited to 5-10% of these plant species. Yet over 2000 have already been reported to contain pest control chemicals¹².

1.4 NATURAL PRODUCTS AS INSECTICIDES

1.4.1 Introduction

Higher plants, microbes and invertebrates have provided lead structures for the development of several commercial insecticides. Three of the four major classes of commercial insecticides are related to the plant metabolites.

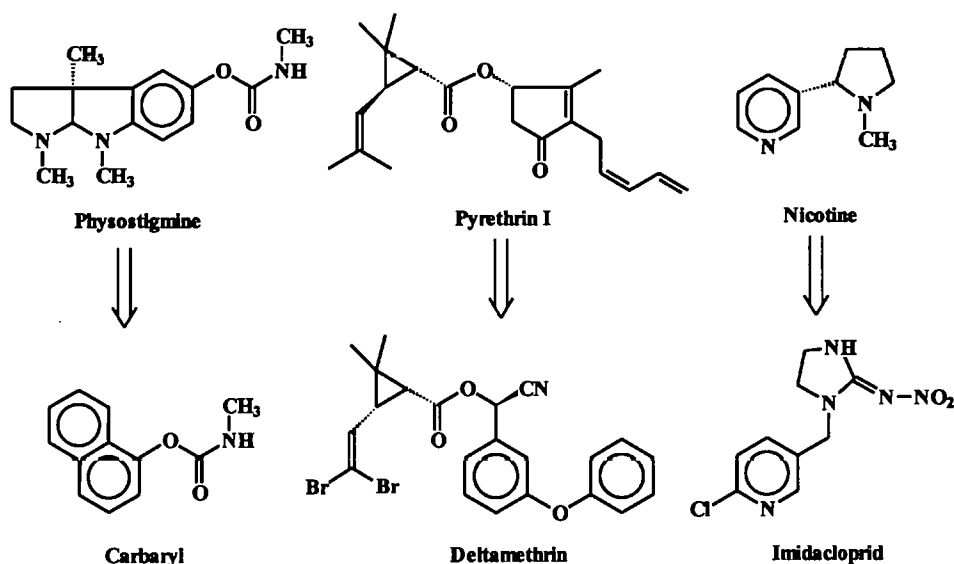


Figure 1.5 : Commercial insecticides related to natural products

1.4.2 Pyrethrins

The powdered dry flower had been used in insect control from ancient times¹³.

1.7 Cinerin I $R_1 = \text{CH}_3$, $R_2 = \text{CH}_3$ **1.8 Cinerin II** $R_1 = \text{CH}_3$, O, C $R_2 = \text{CH}_3$

8

The pyrethrum extract contains six neurotoxic esters¹⁴, pyrethrin I (1.3) and II (1.4), jasmolin I (1.5) and II (1.6) and cinerins I (1.7) and II (1.8) (Figure 1.6). These compounds block voltage-dependent sodium channels in neuromembranes¹⁴. The minimal toxicity of technical pyrethrum to vertebrates (rat oral LD₅₀ - 1500 mg/kg) and its rapid knockdown effects on flying insects have maintained pyrethrum as one of the best active ingredients for the household sector, in particular, for control of the common housefly and mosquitoes.

The pyrethrins are rapidly decomposed by a combination of light and air, and their use was therefore limited mainly to indoor applications. This prompted the development of synthetic pyrethroids starting in the 1940s.

The systematic approach to the development of synthetic pyrethroids was therefore based on the identification of the photolabile centres and the key structural features necessary for insecticidal activity. The constituent acids and alcohols and their simple derivatives are non-insecticidal as Staudinger and Ruzicka demonstrated in their pioneering work¹⁵. Evidence suggested that high insecticidal activity depends on the overall shape of the molecule¹⁶ with certain key structural features appropriately disposed. Other properties such as electron density and polarisability are of secondary importance. Because of the strong dependence of the activity of pyrethroids on structural shape, the effects of structural variation were analysed by segmenting the pyrethrin I as shown in Figure 1.7. In each, the natural component was replaced by different groups

and the activity of the new simple combination examined¹⁷ and this has led to a succession of potent insecticides.

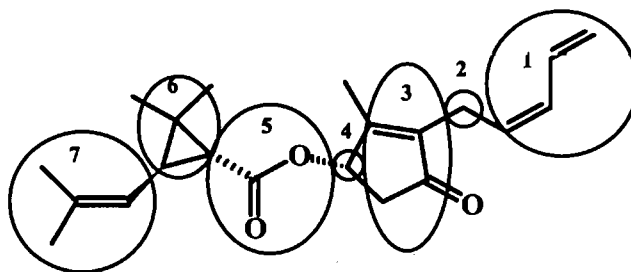


Figure 1.7 : Segmentation scheme for pyrethrin I

Simplification of the diene moiety (Segment 1, Figure 1.7) of pyrethrin I resulted in the discovery of allethrin (1.9) (Figure 1.8) which was the first synthetic pyrethroid with significantly higher activity¹⁸ than the natural extract. However, this modification did not significantly increase photostability and its use was limited to indoor applications. A propargyl analogue of allethrin, prallethrin (1.10) was later found to be one of the most important pyrethroids for household use¹⁹ (Figure 1.8). However, replacement of the diene alcohol by a furfuryl moiety led to a decrease in activity²⁰.

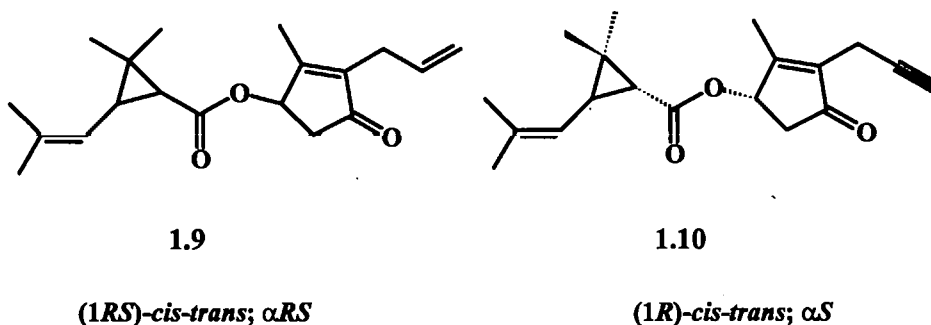


Figure 1.8 : Allethrin and prallethrin

A major advance in pyrethroid research was the discovery of resmethrin (1.11) and the resolved isomer, bioresmethrin (1.12) (Figure 1.9) during the 1960s in which the diene moiety of pyrethrin I was replaced by a phenyl ring and the cyclopentenone moiety by a furan ring^{21, 22}. These compounds showed much higher lethal activity than the pyrethrum extract but were found to be unstable in air and light. Resmethrin and bioresmethrin are now used as non-residual contact sprays in consumer, public health and green house applications.

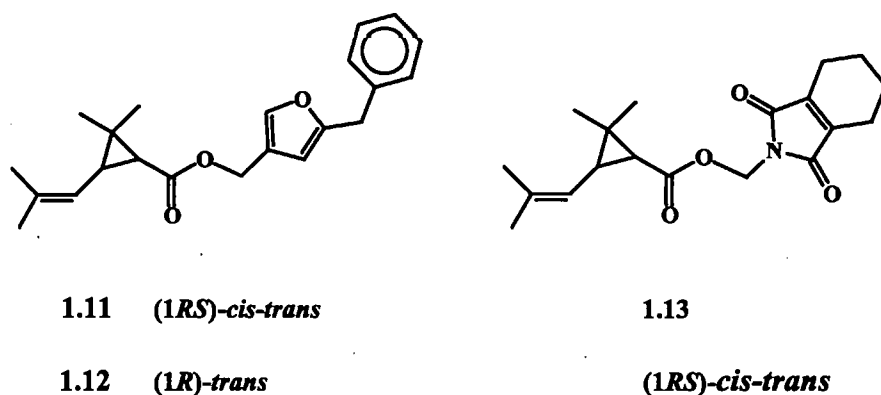


Figure 1.9 : Resmethrin, bioresmethrin and tetramethrin

The discovery that imidomethyl chrysanthemates were also active against housefly led to the development of tetramethrin (1.13) (Figure 1.9). This compound showed exceptionally high knockdown activity against public health pests²³.

The next major breakthrough in the SAR study of pyrethroids was the discovery of the ester of 3-phenoxybenzyl alcohol with chrysanthemic acid, phenothrin (1.14) (Figure 1.10) in the late 1960s²⁴. This pyrethroid was

introduced commercially as (1*R*)-*cis-trans* isomeric mixture, (1*R*)-phenothrin, for household and public health use. It was more stable than resmethrin and showed good toxicity against a wider range of insect species and much lower mammalian toxicity. In the early 1970s another important development was the the insertion of an α -cyano group on the benzylic carbon atom of 3-phenoxybenzyl ester which enhanced the activity more than two fold²⁵. Thus, (1*R*)-cyphenothrin (1.15) (Figure 1.10) is 2-4 times more toxic than phenothrin against a variety of insect pests. These discoveries led to a significant improvement in the level and spectrum of activity of pyrethroids and towards the development of photostable pyrethroids.

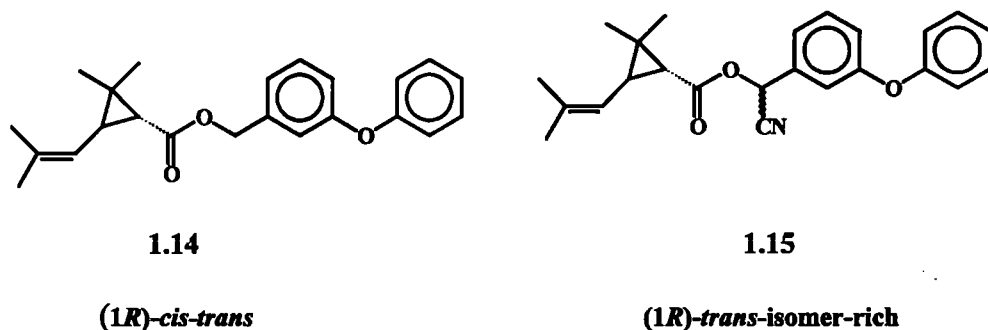


Figure 1.10 : Phenothrin and cyphenothrin

The major breakthrough in SAR studies of pyrethroids came with the discovery of dihalovinyl chrysanthemic acid. Its combination of 3-phenoxybenzyl and α -cyano-3-phenoxybenzyl alcohols led to permethrin (1.16)²⁶, cypermethrin (1.17) and deltamethrin (1.18)²⁷ (Figure 1.11 and 1.12) which were the first photostable pyrethroids in the early 1970s. Permethrin is

at least twenty times more stable in air and light than chrysanthemates such as resmethrin and is more toxic to insects than was predicted by SAR studies.

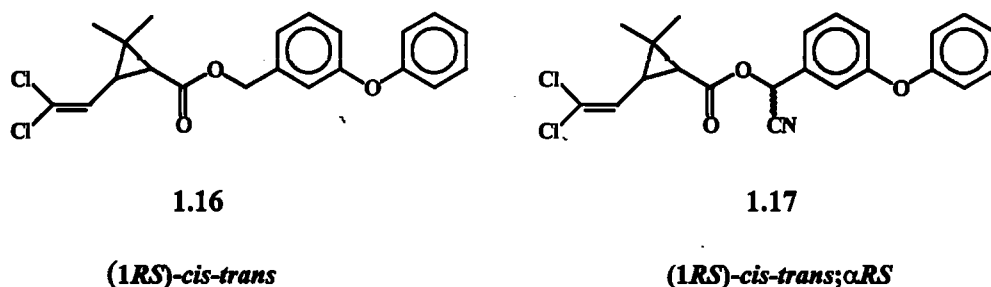


Figure 1.11 : Permethrin and cypermethrin

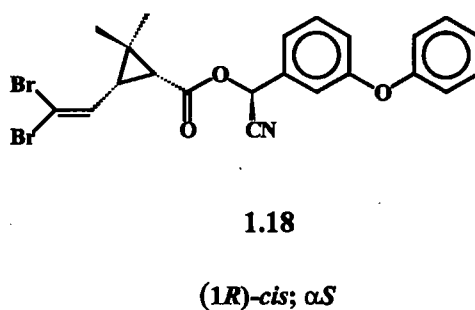
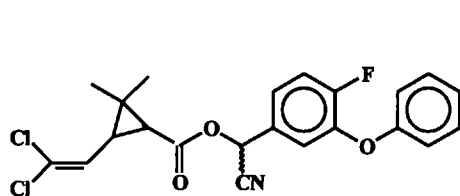


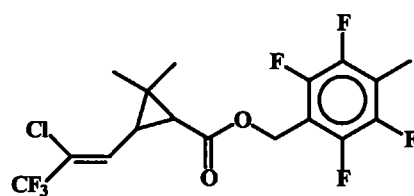
Figure 1.12 : Deltamethrin

The α -cyano-3-phenoxybenzyl alcohol moiety is present in several other commercially important pyrethroids e.g. the 4-fluoro analogue of cypermethrin, cyfluthrin (1.19)²⁸ and a perfluoro analogue, tefluthrin²⁹ (1.20) (Figure 1.13) which is used as a soil insecticide, especially for control of corn root worms.



1.19

(1*RS*)-*cis-trans*; α *RS*

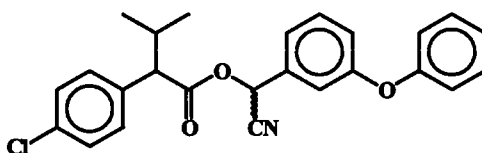


1.20

Z; (1*RS*)-*cis*

Figure 1.13 : Cyfluthrin and tefluthrin

Studies on α -substituted phenylacetates by Japanese researchers who observed toxicity against housefly of the 5-benzyl-3-furylmethyl ester prepared from 2-ethylphenylacetic acid. This work led to the synthesis of fenvalerate (1.21)³⁰ (Figure 1.14), the first non-cyclopropane pyrethroid, which was introduced commercially in 1976 as the racemate.



1.21

RS; α *RS*

Figure 1.14 : Fenvalerate

Following this discovery, a number of other non-cyclopropane pyrethroids such as esfenvalerate (1.22), a fully resolved (*S,S*) isomer and fluvalinate (1.23)³¹ (Figure 1.15) were introduced as agricultural insecticides.

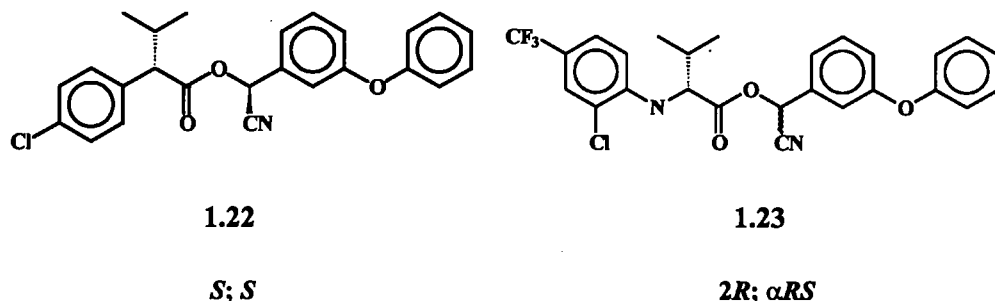


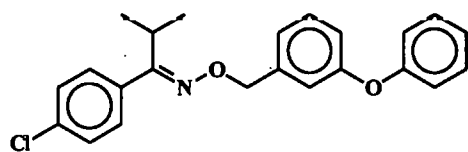
Figure 1.15 : Esfenvalerate and fluvalinate

Further SAR studies indicated that the central ester bond of the original pyrethrins is not essential for insecticidal activity and could be replaced by other suitable groups. The first non-ester pyrethroids discovered were some alkyl aryl ketone oxime *O* ethers such as compounds **1.24a** and **1.24b** (Figure 1.16)^{32, 33}.

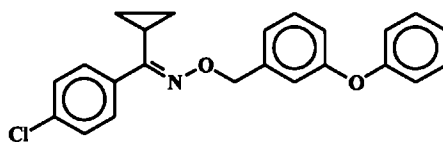
Most examples of non-ester pyrethroids with high insecticidal activity are structurally similar to fenvalerate (**1.21**) (Figure 1.14) but there are considerable differences in which groups can be placed at the isopropyl position in fenvalerate. An important breakthrough was the discovery of etofenprox (**1.24c**) and its analogue MTI-800 (**1.24d**) (Figure 1.16)³⁴. They showed surprisingly high activity, especially against rice pests but with significantly lower fish toxicity when compared with cyclopropane esters.

Changes of gem-dimethyl group in compounds **1.24c** and **1.24d** to a cyclopropyl group have resulted more active compounds such as **1.25a** (Figure 1.16) against houseflies and mustard beetles³⁵. Further SAR studies have

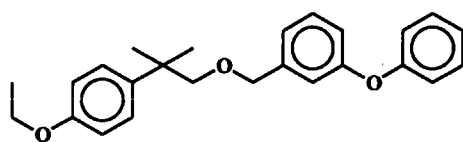
indicated that the gem-dimethyl group is not required for activity and the compound **1.25b** is more active than gem-dimethyl analogues³⁶.



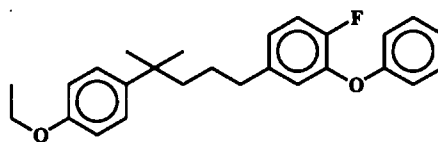
1.24a



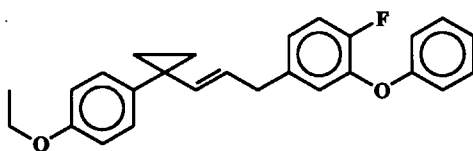
1.24b



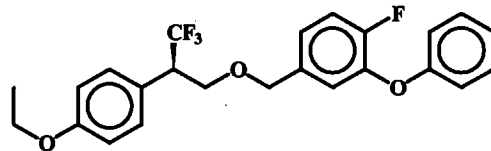
1.24c



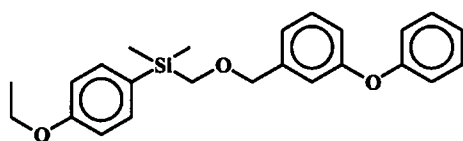
1.24 d



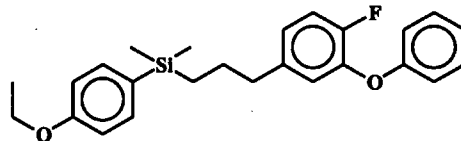
1.25a



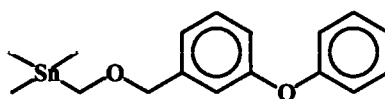
1.25b



1.25c



1.25d



1.25e

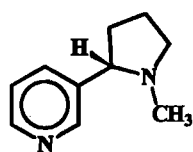
Figure 1.16 : Non-ester pyrethroids

Silicon analogues of etofenprox (1.24c) and MTI-800 (1.24d), compounds, 1.25c and 1.25d (Figure 1.16), respectively, showed good insecticidal activity but they were less active than the corresponding carbon analogues³⁷. However, simple trimethylstanniomethyl ethers such as compound 1.25e (Figure 1.16) have shown surprisingly higher activity against Asiatic rice borer and houseflies than the corresponding carbon analogues¹⁴.

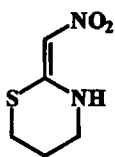
1.4.3 Nicotine and neonicotinoids

Tobacco (*Nicotina tabacum* Linn; Solanaceae) is another equally important insecticidal plant. The aqueous extract of tobacco has been used for plant protection in Europe since the 1600s. The active component of tobacco is nicotine (1.26) (Figure 1.17) which was first, isolated in 1828 from the leaves (1-10% yield)³⁸. Nicotine is effective against a wide range of insects and has a lethal effect through ingestion, contact and fumigation. It affects the nervous system by binding to the acetylcholine receptors³⁹. The use of nicotine has declined significantly over the years due to high mammalian toxicity and low persistence.

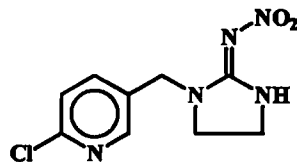
The lengthy struggle to make use of this potent template to synthesise structurally related nicotinoids with the same mode of action was unsuccessful during the early years (1950-1960)³⁸.



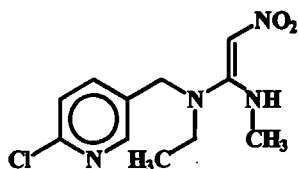
1.26



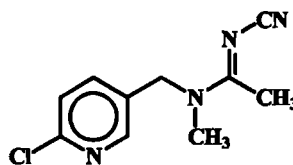
1.27



1.28



1.29



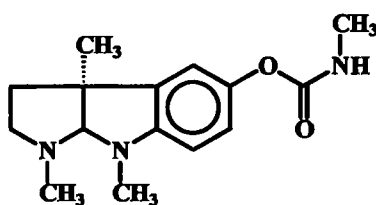
1.30

Figure 1.17 : Nicotine and Neonicotinoids

However, after the discovery of potent insecticidal properties of the nitromethylene analogue, nithiazin (1.27)⁴⁰ (Figure 1.17) in 1979, further studies of nicotine based compounds were resumed. As a result imidacloprid (1.28), the first commercial neonicotinoid (Figure 1.17) was discovered in 1984. Imidacloprid is a systemic insecticide active against plant-sucking insects⁴¹. It also acts as an agonist at the nicotinic acetylcholine receptor⁴² and its mammalian toxicity is much lower than that of nicotine⁴¹. Several other analogues of imidacloprid such as nitenpyram (1.29)⁴³ and acetamiprid (1.30)⁴⁴ with increased insecticidal activity have been discovered (Figure 1.17). Neonicotinoids now form a major new class of insecticides and more compounds of this series are likely to appear in the market.

1.4.4 Carbamates

The first naturally occurring carbamate is physostigmine (1.31) (Figure 1.18), isolated from seeds of the calabar bean, *Physostigma venenosum* Balf. (Leguminosae)⁴⁵. Physostigmine was initially known as a neuro-toxic substance which inhibited the enzyme, acetylcholine esterase³⁹. This prompted the synthesis of numerous analogues of physostigmine for use as cholinergic drugs⁴⁶.



1.31

Figure 1.18 : Physostigmine

The first carbamate esters to exhibit insecticidal properties were analogues of dithiocarbamic acids⁴⁷. These compounds were weakly toxic but several analogues, such as tetraethylthiuram monosulfide were more toxic by contact to aphids. Other carbamates, e.g. thiram, were antifeedants against caterpillars and beetles. However, the high fungicidal activity of these thiocarbamates, together with competition from the more effective organochlorine and organophosphorus insecticides prevented further development of thiocarbamates as insecticides.

Discovery of the insecticidal proprieties of a cycloaliphatic carbamate ester, dimetan (1.32)⁴⁸ (Figure 1.19) in the 1940s led to the further development of carbamates. The most promising compounds were derivatives of heterocyclic enols such as isolan (1.33) and dimetilan (Figure 1.19)^{49, 50}.

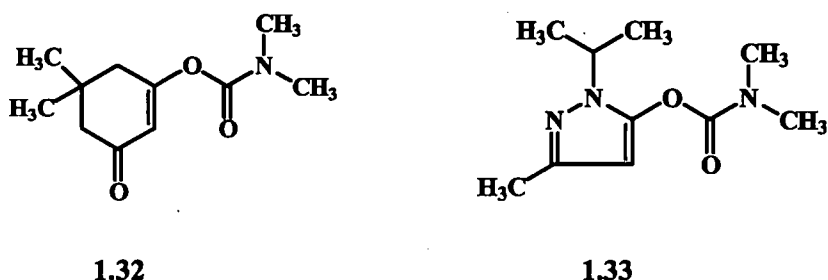


Figure 1.19 : Dimetan and Isolan

Replacement of the enol and dimethylcarbamyl groups in dimetan by aryl and monomethylcarbamyl groups, respectively, led to the development of carbaryl (1.34) (Figure 1.20)⁵¹. The low mammalian toxicity, persistence and the broad-spectrum of activity of carbaryl ensured its use for a long period all over the world.

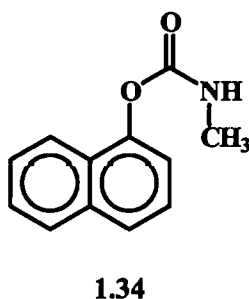


Figure 1.20 : Carbaryl

The potent *in vitro* and poor *in vivo* insecticidal activity of physostigmine was identified much later in 1950s. The poor activity was attributed to the presence of a permanent charge on nitrogen of the amine salts and the quaternary structures which prevented penetration of this molecule through the insect cuticle and nervous system. A series of lipid soluble insecticidal analogues have subsequently been developed⁵².

In the 1960s metolcarb (1.35), carbofuran (1.36) and methomyl (1.37) (Figure 1.21) were developed as more prominent carbamate insecticides but they had rather high mammalian toxicity. A major structural innovation of carbamate chemistry resulted in synthesis of N-methyl carbamate insecticides with reduced mammalian toxicity and with a closer spatial resemblance to acetylcholine⁵³. The result was the discovery of the oxime carbamates in particular aldicarb (1.38) (Figure 1.21), which is a contact and systematic insecticide.

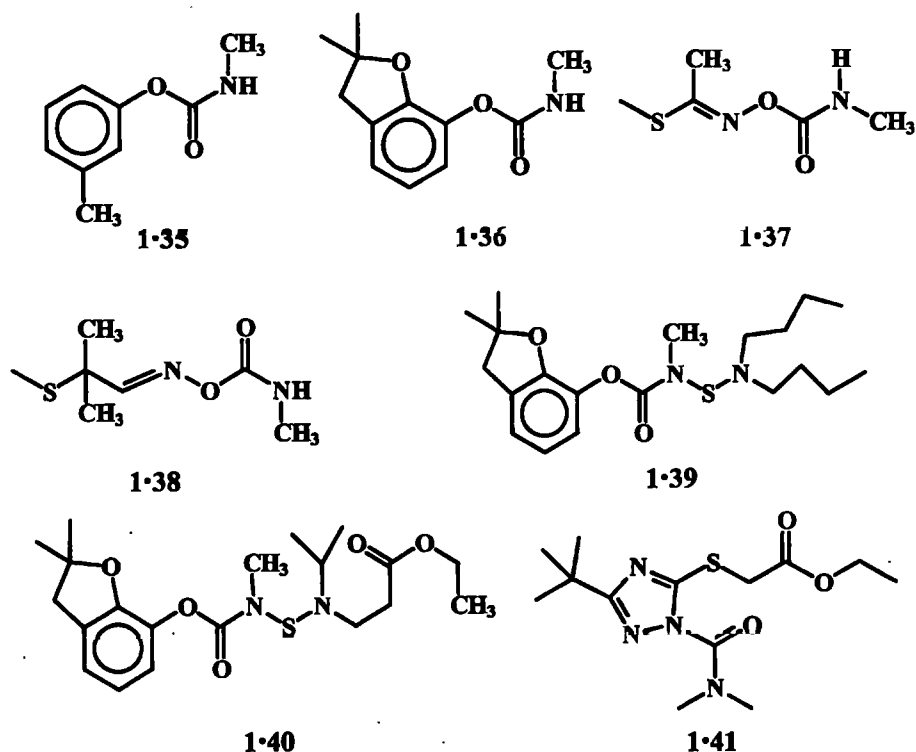
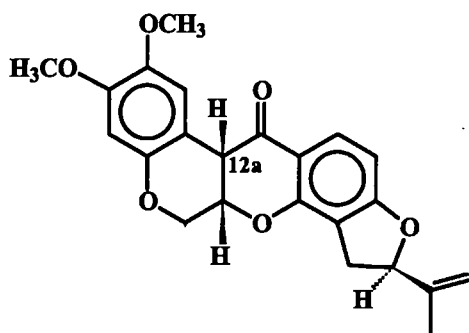


Figure 1.21 : Synthetic carbamates

Further SAR studies led to the development of many other useful carbamate insecticides, such as carbosulfan (1.39), benfuracarb (1.40) and trizamate (1.41) (Figure 1.21)^{54, 55}.

1.4.5 Rotenoids (Isoflavonoids)

“Derris powder” is another well known botanical insecticide which is still popular among gardeners. The active principles of *Derris elliptica* are isoflavonoids called rotenoids (1.42) (Figure 1.22). Rotenoids are also found in *Lonchocarpus*, *Tephrosia* and *Mundulea* species (Leguminosae)³⁸.



1.42

Figure 1.22 : Rotenone

SAR studies on synthetic analogues have identified the key structural features necessary for activity. However, only one analogue in which a CH_3 group is introduced at position 12a was found to be more active than rotenone. The wide use of rotenoids as insecticide has been limited due to their high toxicity towards fish and mammals. The mode of action of this class of compounds has been identified as inhibition of cellular respiration specifically by blocking NADH oxidation⁵⁶.

1.4.6 Ryania

The aerial parts of *Ryania speciosa* (Flacourtiaceae) contain the insecticide ryanodine (1.43) (Figure 1.23). Ryanodine is a complex bridged diterpene heptol³⁸ which has become a commercially successful natural insecticide. Ryanodine acts as a muscle poison by binding to calcium channels in the sarcoplasmic reticulum which allows calcium ions to flow into the cell^{57, 58}. Although mammalian toxicity of ryanodines is high (Rat $\text{LD}_{50} = 750 \text{ mg/kg}$) at

recommended concentrations, it can be considered as safe because technical ryania contains less than 1% of ryanodine⁵⁸.

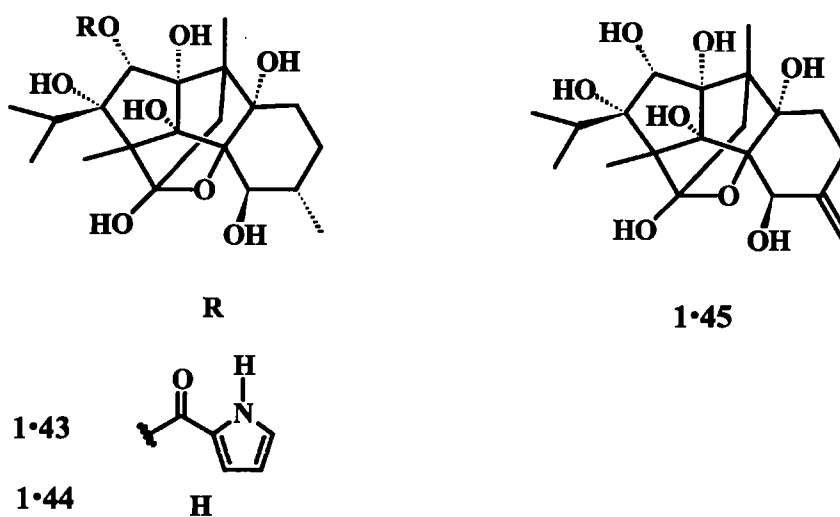


Figure 1.23 : Ryanoids

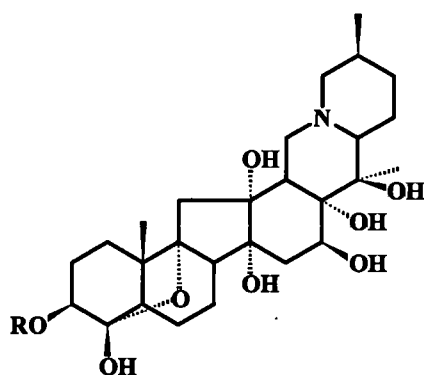
Ryanodol (1.44) and didehydroryanodol (1.45) which are the decomposition products of ryanodine (Figure 1.23) also showed good knockdown activity against houseflies and cockroaches⁵⁷.

1.4.7 Veratrum alkaloids

The powdered seeds of *Veratrum sabadilla* (*Schoenocaulon officinale*; Liliaceae), a native plant of South America, were used for many years as an insecticide¹⁰. The use of sabadilla in the Americas dates back to the 1500s, and the product was used extensively in Europe and USA from the late nineteenth to mid twentieth century¹⁰. It is currently registered in USA for use against a wide range of pests in vegetables, fruits and berry crops. The active

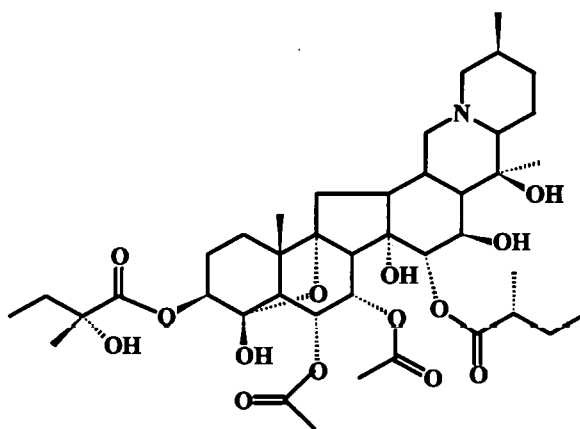
ingredients of *Veratrum* species are a range of alkaloids, including veratridine (1.46), cevadine (1.47), veracevine (1.48) and protoveratrine (1.50) (Figure 1.24)³⁸.

The complexity of the structure has limited SAR studies to simple modifications of the natural compound. Recent SAR studies were based on variations of the acyl group attached to the 3-position of veracevine (1.48). The 3,5-dimethoxy benzoyl derivative was the most active analogue and the activity was found to be almost twice as the natural 3,4-dimethoxy derivative but without an increase in mammalian toxicity.



R

- | | | |
|------|--------------------------------------|------------------------------|
| 1.46 | Veratridine | 3, 4 (MeO) ₂ PhCO |
| 1.47 | Cevadine | Me |
| 1.48 | Veracevine | H |
| 1.49 | 3-(3,5-dimethoxy benzoyl) veracevine | 3, 5 (MeO) ₂ PhCO |



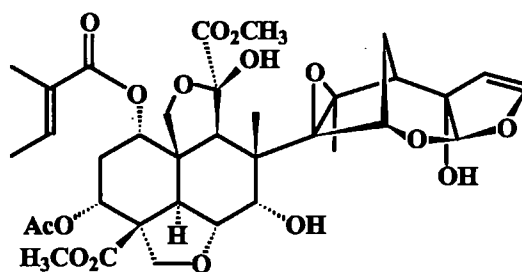
1.50

Figure 1.24 : Veratrum alkaloids

Veratrum alkaloids affect the sodium ion channel³⁹ and show high mammalian toxicity (LD₅₀~12.5 mg/kg). However, the use of powdered seed of *Veratrum* is relatively safe as it contains only 8% alkaloids.

1.4.8 Neem

Neem oil is prepared from the seeds of neem (*Azadirachta indica* A. Juss; Meliaceae) and has been used as a botanical insecticide in India for many years. To date, seventy triterpenoids have been characterised from neem. The most active component, azadirachtin (1.51) (Figure 1.25)⁵⁹ was first isolated in 1968 but the structure was not fully established until 1985⁶⁰. The outstanding antifeedant activity of azadirachtin (1.51) was first demonstrated against the desert locust, *Schistocerca gregaria*⁶¹.



1.51

Figure 1.25 : Azadirachtin

Azadirachtin is also a powerful insect growth regulator which disrupts insect moulting and interferes with reproduction. The major physiological effects in insects are a consequence of reduced synthesis of ecdysteroids⁶². In addition, there is evidence for direct actions of azadirachtin on other organ systems, such as the midgut, and the epidermis, as well as on the gustatory chemosensilla⁶². The use of neem is increasing but the narrow spectrum and slow insecticidal activity has limited its use to selected applications. An

economic synthetic route for azadirachtin has not yet been devised although extensive research in this area has been carried out⁵⁹.

1.4.9 Toosendanin

Toosendanin (1.52), a limonoid, was developed as a botanical insecticide in China over the past decade, from the stem bark of chinaberry trees (*Melia toosendan* and *Melia azadarach*; Meliaceae) (Figure 1.26).

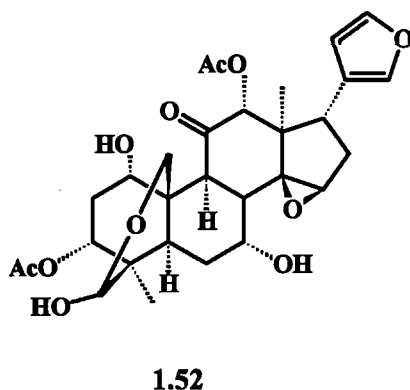


Figure 1.26 : Toosendanin

Toosendanin acts as an antifeedant, stomach poison and a growth inhibitor. It is effective against lepidopteran pests⁶³ but less active than azadirachtin. The mode of action of toosendanin is unknown but it appears to be a presynaptic blocking agent which acts on the neuromuscular system.

1.4.10 Isobutylamides

Many plant species of families Piperaceae, Asteraceae and Rutaceae are known to exhibit insecticidal properties. The principle components are unsaturated isobutylamides³⁸. Pellitorine (1.53) from *Anacyclus pyrethrum* (Asteraceae) was the first insecticidal isobutylamide identified (Figure 1.27). Later, the related compounds affinin (1.54) from *Heliopsis longipes*, pipericide (1.55), dihydropipericide (1.56) and guineensine (1.57) from *Piper nigrum* were isolated (Figure 1.27) and showed greater knockdown and lethal actions against adzuki bean weevil and mosquito larvae⁶⁴.

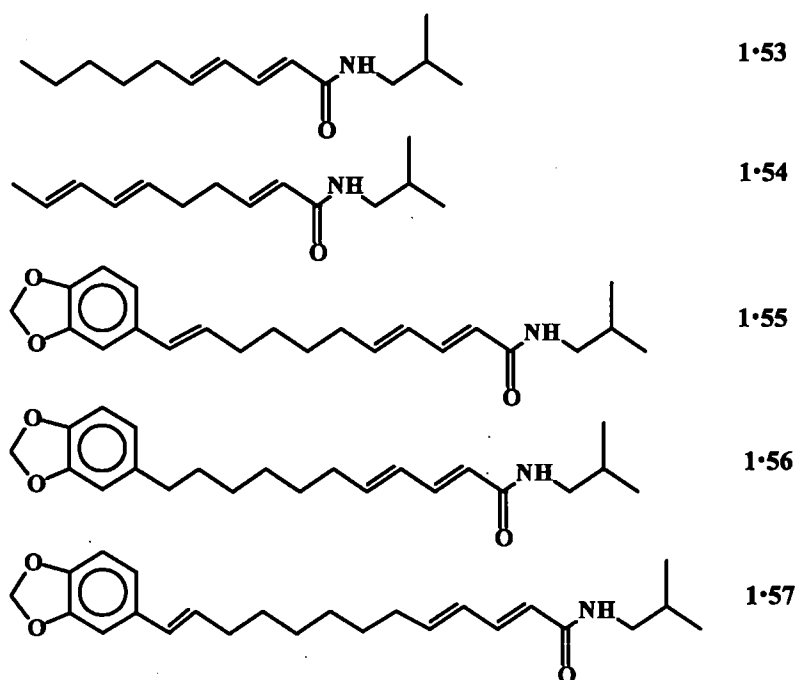


Figure 1.27 : Natural N-alkylamides

Since the natural isobutylamides were not potent enough and had low persistence, some synthetic derivatives have been made. Among them the 3-bromophenyl ether analogue (1.58) (Figure 1.28), in which the halogenated phenoxy ring is substituted for the methylene-dioxy benzyl terminal group, showed about a fifty fold greater insecticidal activity than the natural product⁶⁴. Further structural modifications by shortening the chain length between the phenyl and carbonyl functions and replacing the terminal methylenedioxyphenyl by 6-chloro-2-naphthyl or 3,5-difluorophenyl groups led to the dienamide analogues 1.59 and 1.60 (Figure 1.28) which exhibited high activity against both houseflies and mustard beetles⁶⁵.

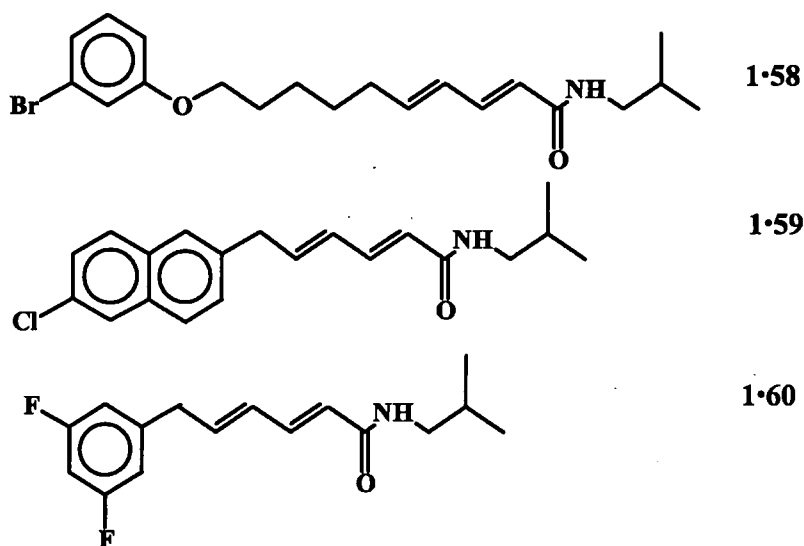
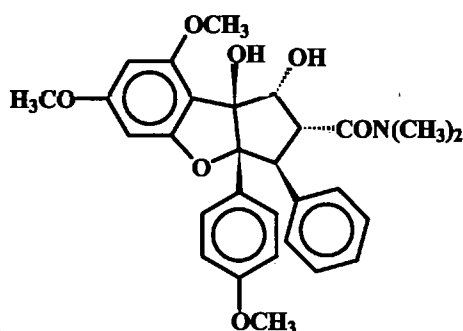


Figure 1.28 : Synthetic N-alkylamides

The isobutylamides act as nerve toxins by blocking the sodium channel⁶⁶. Ultimately the development of isobutylamides as an agricultural product was terminated, as all analogues were unstable in the field.

1.4.11 Benzofurans

Rocaglamide (1.61), a highly substituted benzofuran (Figure 1.29) has been identified as the insecticidal principle in *Aglaia odorata* (Meliaceae), from a plant native to Indo-Malaysia⁶⁷. Recently, several novel rocaglamide derivatives⁶⁸, isolated from *Aglaia roxburghiana* showed high insecticidal activity against *Heliothis virescens*, *Spodoptera littoralis*, and *Plutella xylostella*.



1.61

Figure 1.29 : Rocaglamide

Rocaglamide is a growth inhibitor with activity against variegated cutworms and Asian armyworms⁶⁹. It is a slow acting-toxin, but feeding is severely

inhibited almost immediately, suggesting that central inhibition of the feeding process is the proximate mode-of-action⁷⁰.

1.4.12 Coumarins

Only two coumarins (1.62 and 1.63) from the genus *Mammea* (Guttiferaceae) have been reported to possess insecticidal properties. They all have an acetyl functionality at the 1' position⁷¹ (Figure 1.30).

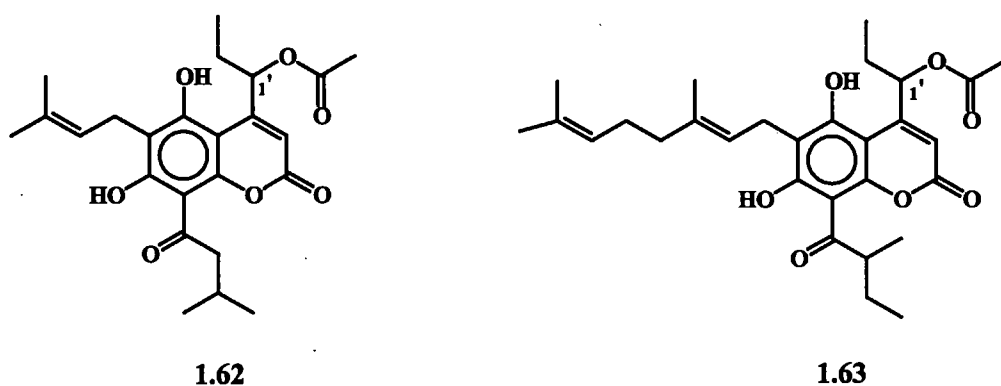
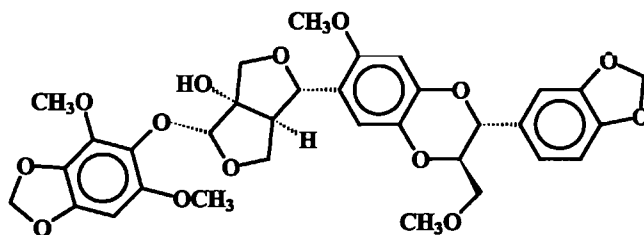


Figure 1.30 : *Mammea* coumarins

1.4.13 Lignans

Haedoxan A (1.64) is an insecticidal sesquilignan, isolated from the roots of *Phryma leptostachya*⁷² (Figure 1.31). It has been shown to be highly active against houseflies by topical application when applied with piperonyl butoxide and several lepidopterous insects by ingestion⁷³.

The lack of activity of other natural and synthetic analogue pointed to the importance of the particular 1,4-benzodioxanyl group unique to Haedoxan A⁷⁴.



1.64

Figure 1.31 : Haedoxan A

1.4.14 Acetogenins

Several plant species of the family Annonaceae (*Annona squamosa*, *Annona muriculata*) have been used as traditional insect control agents in many tropical countries¹⁰. The insecticidal principles isolated from the seed of those plant species are the benzyl isoquinoline alkaloids, annonaines (1.65) and the acetogenins, annonin I (1.66) and asimicin (1.67) (Figure 1.32).

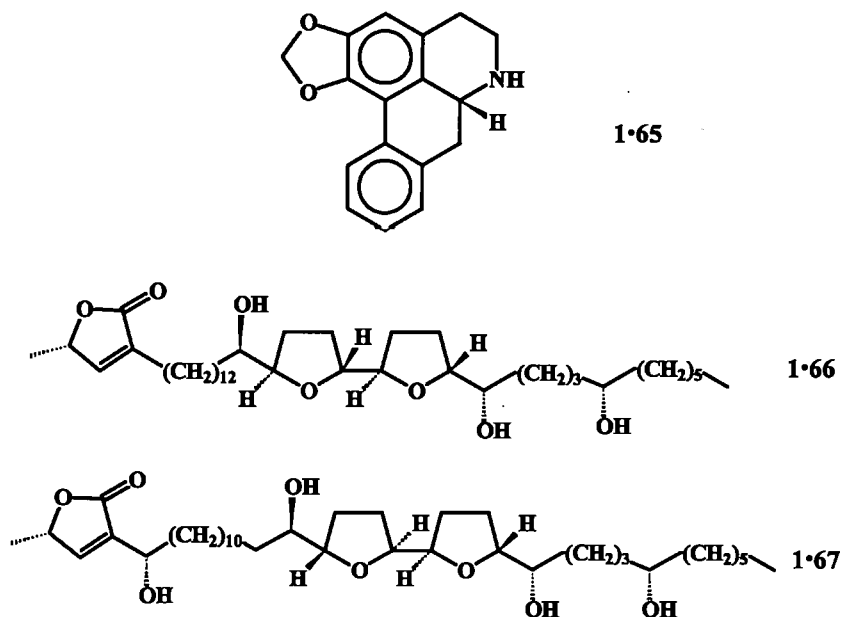


Figure 1.32 : Insecticidal compounds of *Annona* species

Acetogenins are slow acting toxins, effective against the diamondback moth, lady bird beetle and leafhoppers. They inhibit mitochondrial respiration via specific inhibition of NADH-cytochrome reductase, an action analogous to that of rotenone^{75, 76}.

1.4.15 Limonoids

Several limonoids with insecticidal properties have been reported from grapefruit seeds (Rutaceae). These include limonin (1.68) and nomilin (1.69) (Figure 1.33)⁷⁷ which also exhibit antifeedant activity against the Colorado potato beetle (*Leptinotarsa decemlineata*)⁷⁸.

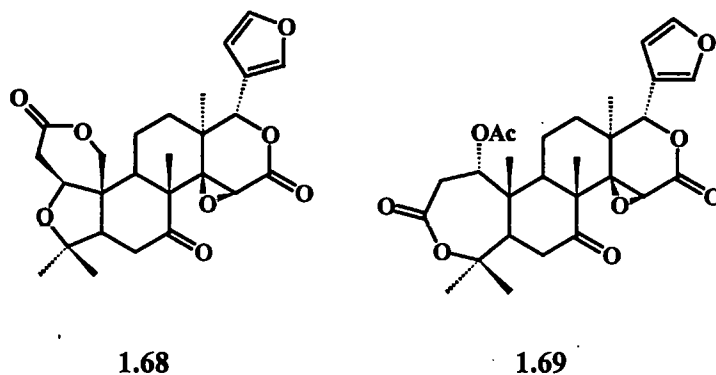


Figure 1.33 : Citrus Limonoids

1.4.16 Sesquiterpenoids

Two known sesquiterpenes PTX (1.70) and argophyllin (1.71) (Figure 1.34) have been reported to be insecticidal⁷⁹.

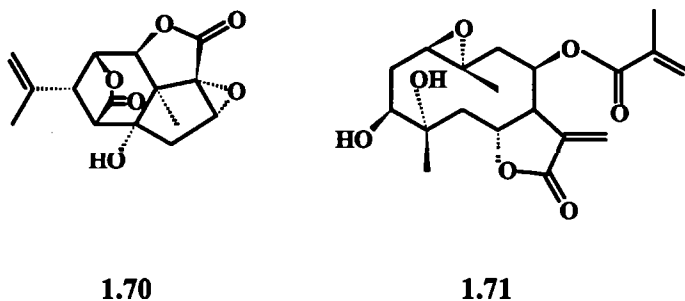


Figure 1.34 : GABA active natural products

The high mammalian toxicities of these compounds prevent their use as botanical insecticides. They act by blocking γ aminobutyric acid (GABA) receptors which are the main neurotransmitters in the neuromuscular junctions and the central nervous systems of insects.

1.4.17 Naphthoquinones

Naturally occurring naphthoquinones have long been known to exhibit a wide range of biological activities. For example the natural products, juglone⁸⁰ is allelopathic and plumbagin⁸¹ is insecticidal. Recently, two related naphthoquinones (Figure 1.35)⁸² have been identified as insecticidal principles from *Calceolaria andina* (Scrophulariaceae).

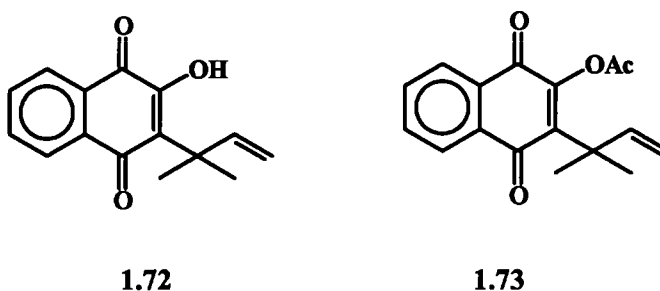


Figure 1.35 : Naphthoquinones

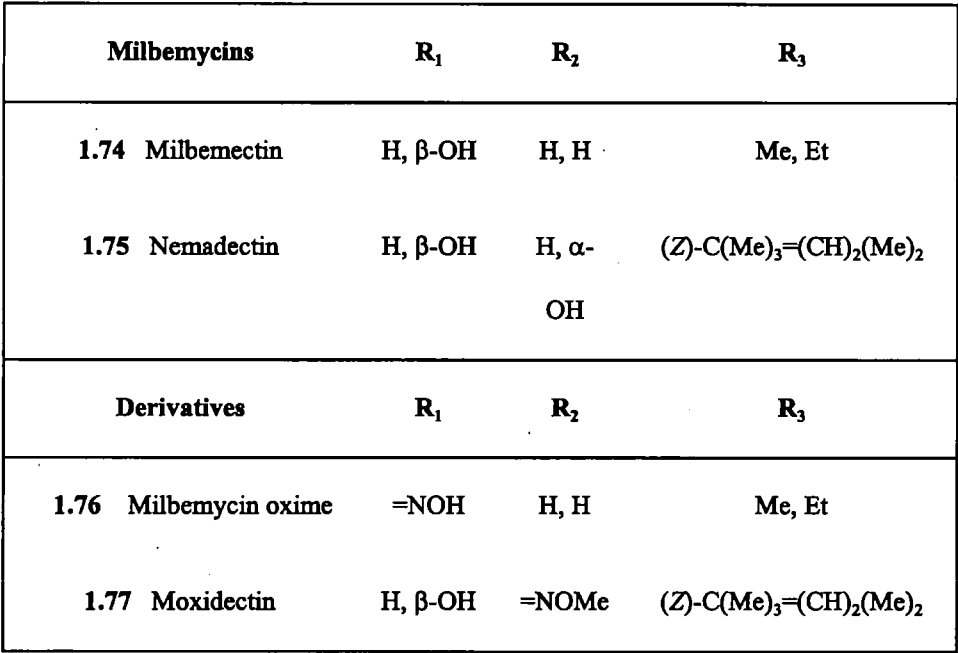
These compounds 1.72 and 1.73 show high insecticidal activity against a range of sucking pests, especially whiteflies (*Bemisia tabaci*), mites (*Tetranychus urticae*) and the peach-potato aphid (*Myzus persicae*). The activity of these compounds is greater than that of the pyrethrins to these species and comparable to the activity of some commercial synthetic insecticides⁸³. Extensive SAR studies based on variations of the alkyl side chain led to the identification of active compounds significantly more active than natural products⁸⁴.

1.4.18 Insecticides of microbial origin

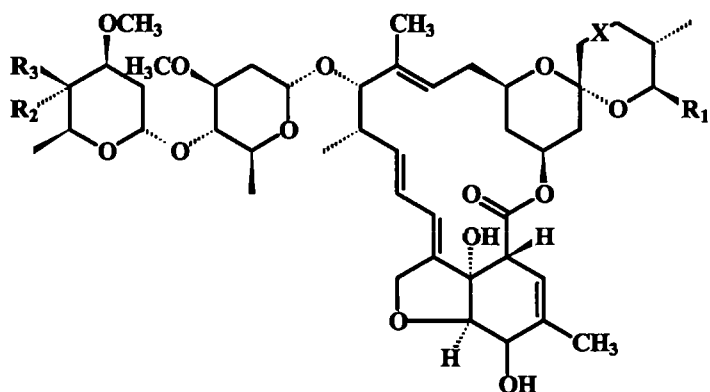
(a) Milbemycins and Avermectins

The milbemycins (1.74-1.77) and avermectins (1.78-1.81) (Figure 1.36 and 1.37) are 16-membered macrocyclic lactones isolated from *Streptomyces* species. They showed very potent anthelmintic and insecticidal activity⁸⁵.

A number of total syntheses of simpler analogues of the natural products were undertaken to define the pharmacophore but none of these attempts led to compounds with high activity. However, semi-synthetic modification of the natural products led to several successful derivatives such as milbemycin oxime, moxidectin, ivermectin, and emamectin (Figure 1.36 and 1.37)⁸⁵ with improved biological properties. Directed biosynthesis was found to be an interesting way to get new highly active analogues such as ivermectin and doramectin^{86, 87, 88}.



Ivermectin is used not only to control agricultural pests but also against animal parasites. In crop protection, abamectin and milbemectin are used as acaricides while abamectin is also active against leaf miners and several lepidopteran pests⁸⁵.



| Avermectins | R ₁ | R ₂ | R ₃ | X |
|-----------------|--------------------|----------------|-------------------|-----------------------------------|
| 1.78 Abamectin | R- ^t Bu | OH | H | CH=CH |
| 1.79 Doramectin | Cyclohexyl | OH | H | CH=CH |
| Derivatives | R ₁ | R ₂ | R ₃ | X |
| 1.80 Ivermectin | R- ^t Bu | OH | H | CH ₂ - CH ₂ |
| 1.81 Enamectin | R- ^t Bu | H | NHCH ₃ | CH=CH |

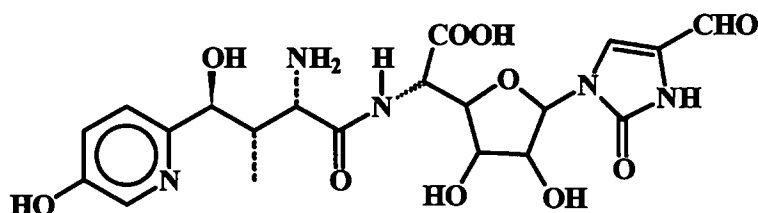
Figure 1.37 : Avermectins

Both milbemycins and avermectins have the same mode of action, the potentiation of glutamate and γ aminobutyric acid (GABA)-gated chloride channel opening⁸⁹.

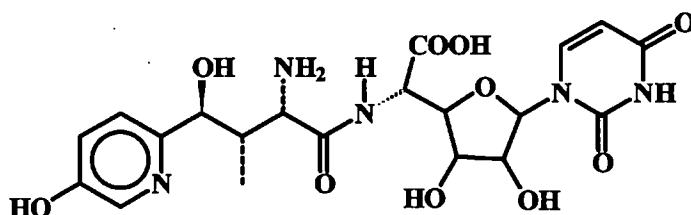
(b) Nikkomycins

Nikkomycins are peptidic nucleoside microbial metabolites isolated from *Streptomyces tendae*⁹⁰. Nikkomycin X (1.82) and Z (1.83), the main members

of this group are acaricidal^{90, 91}. They inhibit chitin synthetase of fungi and insects⁹¹. Since the commercial production of nikkomycin is too expensive, further investigations in this area were suspended.



1.82

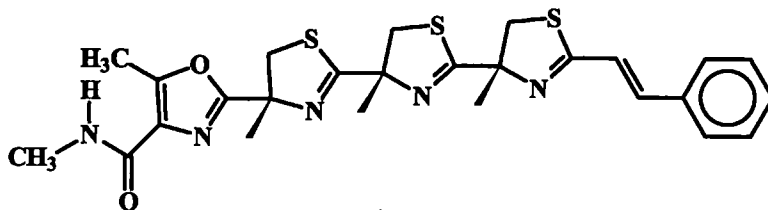


1.83

Figure 1.38 : Nikkomycin X, Nikkomycin Z

(c) Thiagazole

Thiangazole (1.84) (Figure 1.39), a sulphur containing compound was isolated from a culture of myxobacterium, *Polyangium* species as a potent anti-HIV agent⁹². Later, the insecticidal activity of this complicated molecule against *Heliothis virescens* and *Lucilia sericata* was identified⁹³ and total synthesis achieved⁹⁴.

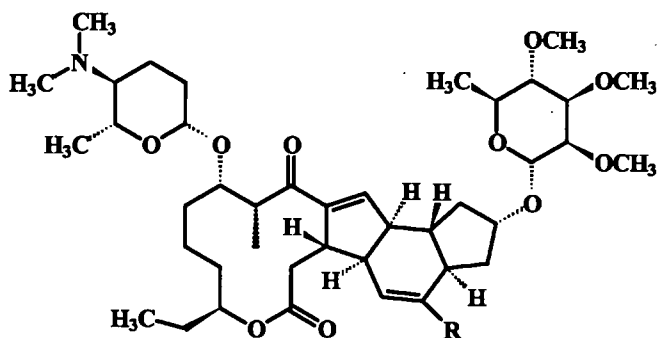


1.84

Figure 1.39 : Thiangazole

(d) Spinosyns

Spinosyns, a new class of highly active natural insecticides were isolated from *Saccharopolyspora spinosa* (actinomycete). The most active spinosyns A (1.85), D (1.86) and several other analogues have been reported^{95, 96} (Figure 1.40). The total synthesis of spinosyn A has been achieved recently⁹⁷ and synthetic analogues showed reduced activity.



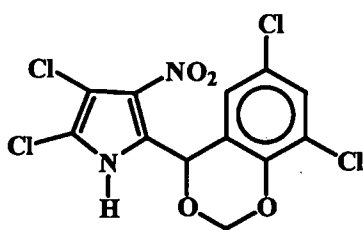
| | |
|----------|------------------|
| Spinosyn | R |
| 1.85 | H |
| 1.86 | Me |
| 1.87 | H : Me = 85 : 15 |

Figure 1.40 : Spinosyns

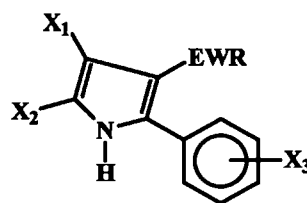
Spinosyns have a novel mode of action. They excite motor neurons but the detailed mechanism is not known yet⁹⁸. There is no cross-resistance to known insecticides. A mixture of spinosyn A and D (85:15), spinosad (1.87), is produced by fermentation and is used to control lepidopteran pests in cotton and is also active against tobacco budworm larvae.

(e) Dioxapyrrolomycin

Dioxapyrrolomycin (1.88) is an insecticidal microbial metabolite isolated from a strain of *Streptomyces fumanus* (Figure 1.41)⁹⁹. It exhibits high activity against tobacco budworms and two-spotted spider mites. However, it also possesses high mammalian toxicity. This compound became the central point for an extensive synthesis program aimed at discovering less complex analogues with improved activity.



1.88



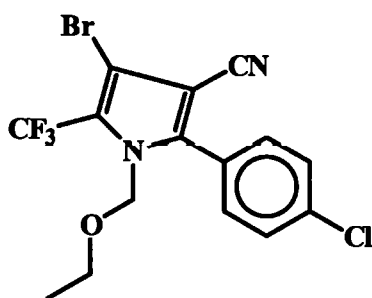
1.89

EWR : CN, NO₂, SO₂CF₃

X₁, X₂, X₃ : Cl, Br, CF₃

Figure 1.41 : Dioxapyrrolomycin and 2-aryl pyrroles

SAR studies led to the identification of the 2-aryl pyrroles (1.89), (Figure 1.41) as a new class of insecticide. The substitution pattern at various positions of the pyrrole nucleus and aryl ring needed for optimal activity has been established; one substituent on the pyrrole ring has to be an electron withdrawing group. Both dioxapyrrolomycin and the 2-aryl pyrroles are uncouplers of oxidative phosphorylation in mitochondria.



1.90

Figure 1.42 : Chlorfenapyr

Chemical modification studies which introduced an ethoxymethyl group on the nitrogen to improve insecticidal activity and to reduce mammalian toxicity resulted in a broadly active compound chlorfenapyr (1.90) (Figure 1.42)¹⁰⁰. Chlorfenapyr was developed as a new insecticide to control mainly lepidopteran insects.

1.5 ASSAYS FOR DETECTION OF INSECTICIDAL NATURAL PRODUCTS

The availability of an appropriate bioassay system is a key requirement for the identification of insecticidal compounds. In establishing such bioassays, several major aspects need careful consideration i.e. life stages (adults, larvae and eggs), use of model insect species, application routes (e.g. topical, residual, vapour and systemic), throughput, decision on threshold activity and *in vivo* or *in vitro* screens. These considerations are inter-related and affect the ability of the systems to identify reliably low levels of activity against relevant insects which could be due to small amounts of highly active compounds. Significance of such considerations and their impact on the discovery process can be illustrated by following examples: development of synthetic pyrethroids based on houseflies and mustard beetles at Rothamsted³⁵, the comparative SAR studies of acetogenins based on Brine shrimp³⁸ and the discovery of previously un-detected naphthoquinones based on residual assays⁸⁴. In this research, priority was given for insecticidal bioassays based on five species, mustard beetles (*Phaedon cochleariae*), housefly (*Musca domestica*), larvae of the diamondback moth (*Plutella xylostella*), whitefly (*Bemisia tabaci*) and mites (*Tetranychus urticae*). All the assays entailed direct contact of the test compound with the test insect species. Although mustard beetles and houseflies are not commercially important agricultural pests, their usefulness as relevant models was recognised in the discovery of synthetic pyrethroids²¹.

CHAPTER 2

RESULTS AND DISCUSSION

2.1 INTRODUCTION

Over 400 plant species have been collected from different geographical locations of Sri Lanka in an attempt to search for new lead insecticidal compounds as part of the agreement between IACR-Rothamsted and the University of Colombo, Sri Lanka (1991-1998). Sequential extraction of these plant materials was carried out with organic solvents (petroleum ether, ethyl acetate and ethanol) and the extracts were tested for insecticidal activity against a range of resistant and susceptible insect species, mustard beetle (*Phaedon cochleariae*), housefly (*Musca domestica*), larvae of diamondback moth (*Plutella xylostella*), whitefly (*Bemisia tabaci*) and mites (*Tetranychus urticae*). Several plant species showed promising insecticidal activity and are summarised in Table 2.1. The work embodied in this thesis endeavours to explore the chemistry and insecticidal activity of compounds isolated from the four plant species highlighted in Table 2.1.

Table 2.1 : Insecticidal Activity of Sri Lankan Plants

| Plant species | Family | MB* | HF* | PX* | BT† | TU* |
|-----------------------------------|--------------|-----|-----|-----|-----|-----|
| <i>Acronychia pedunculata</i> | Rutaceae | 100 | 100 | WT | WT | WT |
| <i>Pleurostyliia opposita</i> | Celastraceae | 100 | WT | WT | WT | NT |
| <i>Walsura piscida</i> | Meliaceae | 100 | NT | NT | NT | NT |
| <i>Alseodaphne semicarpifolia</i> | Lauraceae | WT | 89 | NT | NT | NT |

Table contd.

| | | | | | | |
|-------------------------------|----------------|-----|-----|----|----|----|
| <i>Aegle marmelos</i> | Rutaceae | 100 | 79 | 20 | NT | NT |
| <i>Atalanta zeylanica</i> | Rutaceae | 90 | NT | - | 42 | - |
| <i>Feronia limonia</i> | Rutaceae | 60 | NT | 20 | - | NT |
| <i>Choroxylon swiotonia</i> | Rutaceae | NT | 50 | NT | NT | NT |
| <i>Excoecaria agallocha</i> | Euphorbiaceae | 100 | 79 | 20 | NT | NT |
| <i>Annona squamosa</i> | Annonaceae | 100 | NT | 70 | NT | - |
| <i>Moringa oleifera</i> | Moringaceae | 80 | NT | 20 | NT | - |
| <i>Piper longum</i> | Piperaceae | 100 | NT | NT | NT | - |
| <i>Anacardium occidentale</i> | Anacardiaceae | - | 65 | - | - | - |
| <i>Acacia leucophloea</i> | Leguminosae | 55 | NT | WT | - | - |
| <i>Tephrosia purpurea</i> | Leguminosae | 100 | NT | NT | - | - |
| <i>Tagetes erecta</i> | Compositae | 90 | - | - | - | - |
| <i>Leucas zeylanica</i> | Labiatae | 70 | NT | NT | - | - |
| <i>Anamirta cocculus</i> | Menispermaceae | 60 | NT | NT | NT | - |
| <i>Ardisia humilis</i> | Myrsinaceae | 85 | 100 | 30 | - | WT |
| <i>Hortonia angustifolia</i> | Monimiaceae | NT | 66 | NT | NT | NT |
| <i>Loca indica</i> | Leeaceae | 65 | NT | NT | NT | - |

Insecticidal activity is given as % mortality (^o20 µg/insect, ⁺10 µg/insect, [†]2000 ppm, [♥]1000 ppm)

NT: non toxic, WT: weakly toxic (<20%). MB : mustard beetle (*Phaedon cochleariae*),

HF: housefly (*Musca domestica*), PX: diamondback moth larvae (*Plutella xylostella*),

BT : whitefly (*Bemisia tabaci*), TU: mites (*Tetranychus urticae*).

2.2 ISOLATION AND CHARACTERISATION OF INSECTICIDAL COMPOUNDS FROM *ACRONYCHIA PEDUNCULATA*

2.2.1 Introduction

Acronychia pedunculata (L.) Miq. (Rutaceae) is a small sized tropical tree, widely distributed in Sri Lanka. Members of the family Rutaceae are known to exhibit a wide spectrum of biological activities including pharmacological, insecticidal, allelopathic, antifungal and antibacterial activities^{38, 77, 101}.

A. pedunculata is a known medicinal plant used in folk medicine in Asia¹⁰². Extracts of stem and root bark of this plant showed fungicidal¹⁰³ and cytotoxic¹⁰⁴ activities. However, to date, no insecticidal activity has been reported for the genus *Acronychia*.

Previous work on the genus *Acronychia* has led to the isolation and characterisation of a series of prenylated aryl ketones¹⁰⁵⁻¹⁰⁹, furoquinoline alkaloids^{108, 110}, furanocoumarins¹⁰⁸ and triterpenes¹⁰⁸ from the stem bark, leaves, root and fruits (Figure 2.1 and 2.2). Acrovestone which is an aryl ketone dimer showed anti-tumour activity in human KB cell line at 0.8 µg/ml¹⁰⁴.

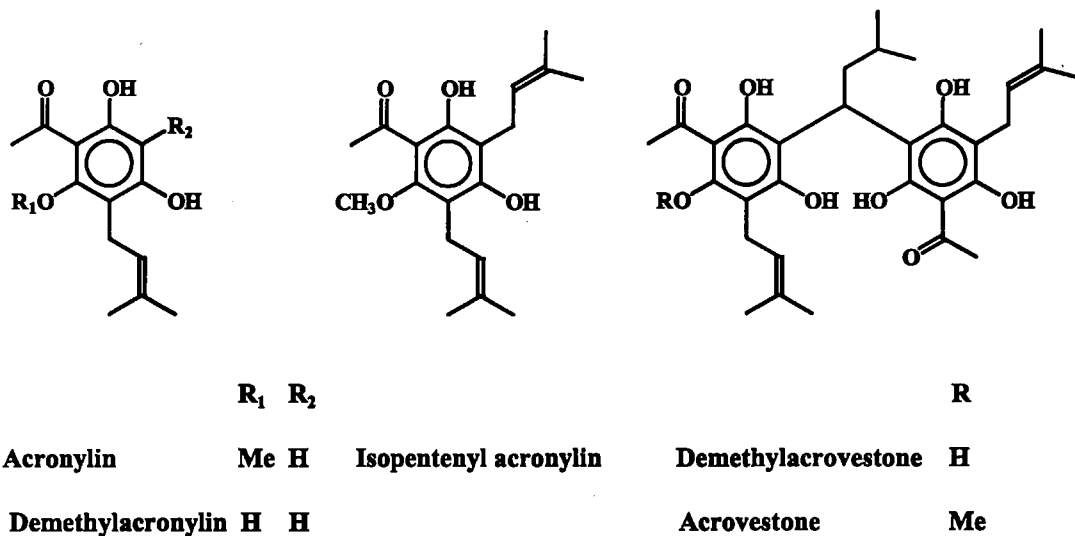


Figure 2.1 : Prenylated aryl ketones

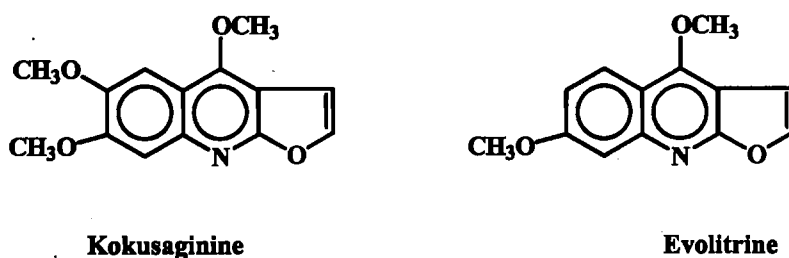


Figure 2.2 : Furoquinoline alkaloids

Preliminary bioassay studies of the organic extracts of the stem bark of *A. pedunculata* showed that they exhibited high insecticidal activity against mustard beetles (*Phaedon cochleariae*) and houseflies (*Musca domestica*) (Table 2.2) and prompted further investigations.

Table 2.2 : Insecticidal activity of *Achronychia pedunculata*

| Extract | MB [*] | HF [*] | PX ⁺ | BT [‡] | TU [▼] |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Petroleum ether | 100 | 100 | WT | WT | WT |
| Ethyl acetate | NT | NT | NT | NT | NT |
| Ethanol | NT | NT | NT | NT | NT |

Insecticidal activity is given as % mortality (^{*}20 µg/insect, ⁺10 µg/insect, [‡]2000 ppm, [▼]1000 ppm).

NT: non toxic, WT: weakly toxic (<20%). MB : mustard beetle (*Phaedon cochleariae*),

HF: housefly (*Musca domestica*), PX : larvae of diamondback moth (*Plutella xylostella*),

BT : whitefly (*Bemisia tabaci*), TU : mites (*Tetranychus urticae*).

2.2.2 Isolation of insecticidal compounds

Sequential extraction of the stem bark of *A. pedunculata* showed that the insecticidal activity was concentrated in the non-polar petroleum ether extract (Table 2.2). The bio-assay guided fractionation of the petroleum ether extract by dry column flash chromatography gave a major insecticidal fraction containing one compound. Recrystallisation of this fraction in methanol, allowed the isolation of compound 1 as a yellow crystalline solid (Figure 2.3).

2.2.3 Characterisation of compound 1

Compound 1 was isolated as a bright yellow crystalline solid (m.p. 142-144 °C). The Electron Impact (EI) mass spectrum of 1 displayed the molecular ion at m/z 554 indicating the molecular formula to be $C_{32}H_{42}O_8$. The presence of an acetophenone carbonyl moiety was confirmed by a characteristic strong absorption band at 1608 cm^{-1} in the IR spectrum.

The ^1H NMR spectrum indicated signals for two prenyl [δ 1.68 (3H, s), 1.75 (3H, s), 1.77 (3H, s), 1.82 (3H, s), 3.32 (2H, d), 3.34 (2H, d) and 5.21 (2H, t)], one isobutyl [δ 0.84 (6H, br s), 1.41 (1H, m), 2.24 (2H, m) and 4.74 (1H, t)], one 2,4,6-trihydroxyacetophenone and one 2-methoxy-4,6-dihydroxyacetophenone groups.

The ^{13}C NMR spectrum showed the presence of thirty two carbons in the molecule (Table 2.3). Multiplicities of the carbons were determined on the basis of the DEPT spectrum, indicating nine methyl, three methylene, four methine and sixteen quaternary carbons to be present in the molecule. The direct connectivities of carbons and their respective protons were established from analysis of the ^{13}C - ^1H COSY spectrum.

On the basis of the foregoing evidence which was in agreement with the reported NMR data (Table 2.3)¹⁰⁴, compound 1 was identified as acrovestone.

Acrovestone has previously been isolated from *Acronychia vestita*¹⁰⁵,

Acronychia laurifolia and *Acronychia pedunculata*¹⁰⁶ and the structure has been derived by both chemical¹⁰⁵ and spectroscopic methods¹⁰⁴.

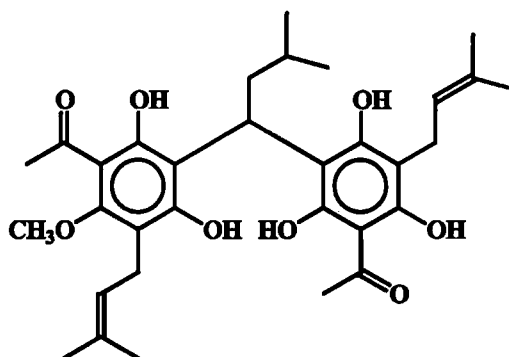


Figure 2.3 : Compound 1

Acrovestone (1) is a prenylated polyphenol, first reported in 1961, and the structure was confirmed by single-crystal X-ray analysis which provided details of the solid state geometry¹¹¹. However, a detailed 2D NMR study of acrovestone has not been reported. Hence, a series of 2D NMR experiments was carried out in the present study (Table 2.3 and 2.4).

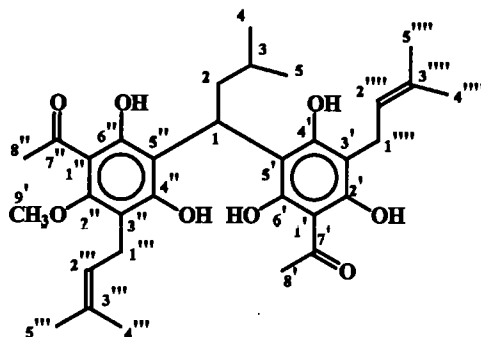


Table 2.3 : Comparison of ^{13}C and ^1H NMR spectral data of compound 1
with those reported for acrovestone in CDCl_3

| Position | Reported δ_{C} | Observed δ_{C} | Reported δ_{H} (J) | Observed δ_{H} (J) |
|------------|------------------------------|------------------------------|----------------------------------|----------------------------------|
| 1 | 28.6 | 28.8 | 4.74, t (7.7) | 4.74, t (7.7) |
| 2 | 39.4 | 39.6 | 2.15, m | 2.24, m |
| 3 | 27.0 | 27.2 | 1.41, m | 1.41, m |
| 4 | 22.5 | 22.5 | 0.87, d (6.4) | 0.84, br s |
| 5 | 22.7 | 22.7 | 0.87, d (6.4) | 0.84, br s |
| 1' 1'' | 116.7, 113.0 | 116.8, 113.2 | - | - |
| 2' 2'' | 162.6, 158.3 | 162.7, 158.3 | - | - |
| 3' 3'' | 106.2, 104.7 | 106.3, 104.9 | - | - |
| 4' 4'' | 160.2, 158.3 | 160.3, 158.4 | - | - |
| 5' 5'' | 108.2, 108.6 | 108.3, 108.6 | - | - |
| 6' 6'' | 160.8 | 160.8, 160.9 | - | - |
| 7' 7'' | 204.2, 204.3 | 204.2, 204.3 | - | - |
| 8' 8'' | 32.6, 30.7 | 32.6, 30.6 | 2.67, 2.71, s | 2.67, 2.71, s |
| 9' | 62.6 | 62.6 | 3.71, s | 3.71, s |
| 1''' 1'''' | 22.2, 23.1 | 22.3, 23.2 | 3.30, 3.40, d (6.6) | 3.32, 3.34, d (6.6) |
| 2''' 2'''' | 121.6, 123.1 | 121.6, 123.2 | 5.20, t (6.6) | 5.21, t (6.6) |
| 3''' 3'''' | 131.8, 136.7 | 131.6, 136.7 | - | - |
| 4''' 4'''' | 17.9, 18.0 | 17.9, 18.0 | 1.69, 1.77, s | 1.68, 1.75, s |
| 5''' 5'''' | 25.7, 25.8 | 25.6, 25.7 | 1.77, 1.84, s | 1.77, 1.82, s |
| 2'-OH | - | - | 15.70, br s | 15.70, br s |
| 4'-OH | - | - | 6.50, br s | 6.50, br s |
| 6'-OH | - | - | 10.05, br s | 10.20, br s |
| 4''-OH | - | - | 9.34, br s | 9.30, br s |
| 6''-OH | - | - | 15.65, s | 15.58, s |

Chemical shift values are in ppm. Coupling constants (J values) in parentheses are in Hz.

^1H NMR spectrum is recorded at 50°C .

Table 2.4 : ^{13}C , DEPT and ^{13}C - ^1H COSY spectral data for compound 1 in CDCl_3

| Position | δ_{C} | DEPT | ^{13}C - ^1H COSY | ^1H - ^1H COSY |
|-------------|---------------------|---------------|-------------------------------------|----------------------------------|
| 1 | 28.8 | CH | 4.74 | 2.24 |
| 2 | 39.6 | CH_2 | 2.24 | 4.74 |
| 3 | 27.2 | CH | 1.41 | 0.84 |
| 4 | 22.5 | CH_3 | 0.84 | 1.41 |
| 5 | 22.7 | CH_q | 0.84 | 1.41 |
| 1', 1'' | 116.8, 113.2 | C_q | - | - |
| 2', 2'' | 162.7, 158.3 | C_q | - | - |
| 3', 3'' | 106.3, 104.9 | C_q | - | - |
| 4', 4'' | 160.3, 158.4 | C_q | - | - |
| 5', 5'' | 108.3, 108.6 | C_q | - | - |
| 6', 6'' | 160.8, 160.9 | C_q | - | - |
| 7', 7'' | 204.2, 204.3 | C_q | - | - |
| 8', 8'' | 32.6, 30.6 | CH_3 | 2.67, 2.71 | - |
| 9' | 62.6 | CH_3 | 3.71 | - |
| 1''', 1'''' | 22.3, 23.2 | CH_2 | 3.32, 3.34 | 5.21 |
| 2''', 2'''' | 121.6, 123.2 | CH | 5.21 | 3.32, 3.34 |
| 3''', 3'''' | 131.6, 136.7 | C_q | - | - |
| 4''', 4'''' | 17.9, 18.0 | CH_3 | 1.68, 1.75 | 5.21, 3.32 |
| 5''', 5'''' | 25.6, 25.7 | CH_3 | 1.77, 1.82 | 5.21, 3.34 |

Chemical shift values are in ppm.

The ^1H NMR spectrum (400 MHz, CDCl_3 , 25°C) showed ten sets of peaks in different chemical environments and their multiplicities were difficult to assign at this field strength. For instance, the four benzylic protons which resonated at δ 3.32 and 3.34 appeared as a broad singlet instead of a double doublet at 25°C . The two protons resonating at δ 2.24 of the isovaleryl moiety were also a broad singlet rather than a double doublet at 25°C .

This peak broadening may be due to restricted rotation in the molecule. In support of this, the ^1H NMR spectrum recorded at 50°C exhibited higher resolution when compared with those recorded at room temperature. The ^1H - ^1H COSY correlations of acrovestone were also in good agreement with the assigned structure.

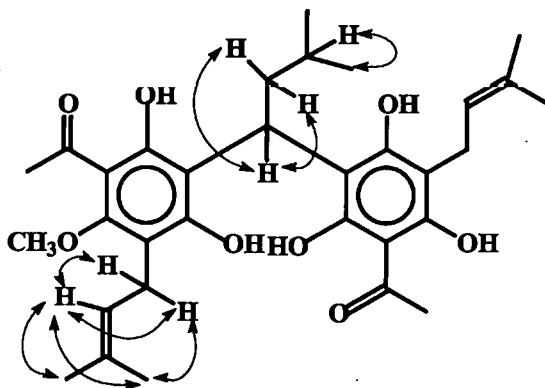


Figure 2.4 : ^1H - ^1H COSY correlations for acrovestone

2.3 ISOLATION AND CHARACTERISATION OF INSECTICIDAL COMPOUNDS FROM *PLEUROSTYLIA OPPOSITA*

2.3.1 Introduction

Pleurostyliya opposita (Wall) Alston (Celastraceae) is a commercially important tropical timber tree found in the dry zone of Sri Lanka. Previous work¹¹²⁻¹¹⁷ on the genus *Pleurostyliya* has led to the isolation of limited phytochemical constituents such as steroids, triterpenes and macrocyclic spermidine alkaloids but the biological activity of these constituents has not been reported. However, insecticidal, antifeedant and narcotically active compounds have been isolated from other genera within the Celastraceae¹¹⁸⁻¹²⁰

Table 2.5 : Insecticidal activity of *Pleurostyliya opposita*

| Insect species | Stem bark | | | Leaves |
|----------------|-----------|-----|----|--------|
| | A | B | C | C |
| MB* | 100 | 100 | NT | NT |
| HF* | WT | NT | NT | NT |
| PX† | WT | NT | WT | NT |
| BT‡ | 20 | NT | NT | - |
| TU¶ | NT | NT | NT | - |

Insecticidal activity is given as % mortality (* 20 µg/insect, †10 µg/insect, ‡2000 ppm, ¶1000 ppm).

NT: non toxic, WT : weakly toxic (<20%). MB : mustard beetle (*Phaedon cochleariae*),

HF: housefly (*Musca domestica*), PX : larvae of diamondback moth (*Plutella xylostella*),

BT: whitefly (*Bemisia tabaci*), TU : mites (*Tetranychus urticae*).

A : petroleum ether, B : ethyl acetate, C : ethanol.

The insecticidal activity of extracts of *P. opposita*, collected in Sri Lanka was first discovered at Rothamsted in another Ph.D investigation and are summarised in Table 2.5¹²¹. The insecticidal principles have been tentatively identified as a series of novel macrocyclic sesquiterpene polyol ester alkaloids, compounds 2.1 and 2.2 (Figure 2.5)¹²¹. They are homologues of known macrocyclic sesquiterpene alkaloids (Figure 2.6)^{118, 122} in which the 6,6 ring skeleton was replaced by a 6,7 ring skeleton. Characterisation of the compounds 2.1-2.3 was based extensively on spectroscopic data. However, the structural identification of 2.1-2.3 using chemical and X-ray analysis has not been done.

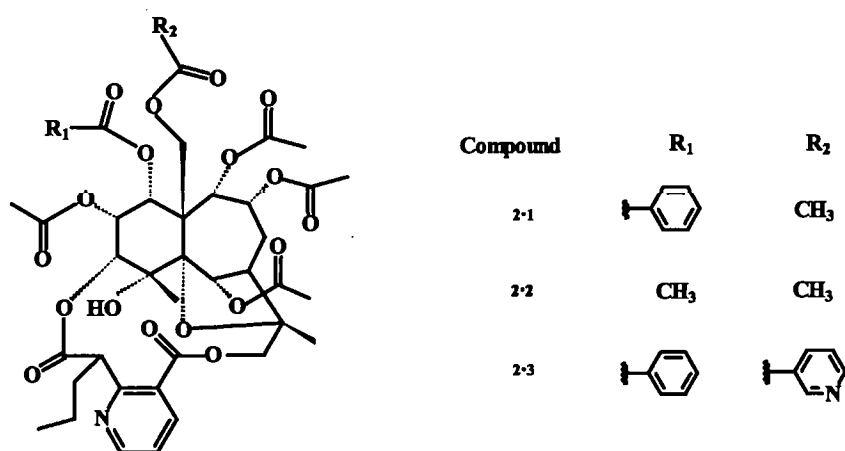
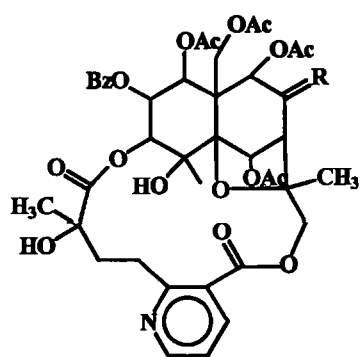
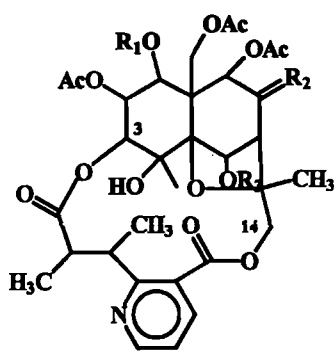
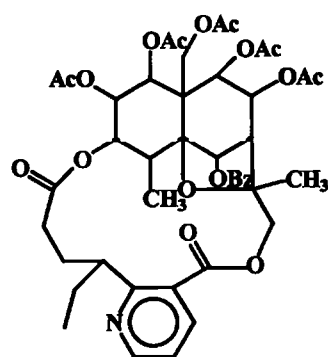
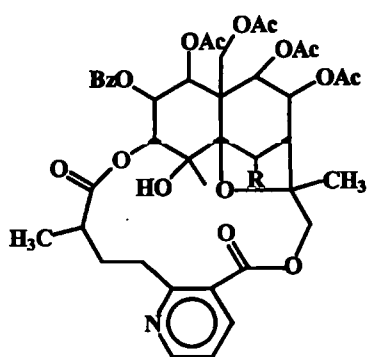


Figure 2.5 : Compounds 2.1, 2.2 and 2.3

Subsequent consideration of the possible biosynthetic pathways¹¹⁸ to the compounds 2.1-2.3 prompted reinvestigation of the proposed structures (Figure 2.5) in the present study.



| | R ₁ | R ₂ | R ₃ | | R |
|---------------------------|----------------|----------------|----------------|-------------------|------------|
| Euonymine | Ac | β-OAc | Ac | Wilfordine | OAc |
| Mayteine | Bz | β-OAc | Ac | Alatamine | OBz |
| Evonine | Ac | O | Ac | | |
| Cangorinine E-1 Ac | β-OAc | | Bz | | |



| | R | |
|-------------------------|------|-----------------|
| Cangorinine W-I | OBz | Cassinin |
| Cangorinine W-II | ONic | |
| Wilforine | OAc | |

Figure 2.6 : Sesquiterpene polyol ester alkaloids

2.3.2 Isolation of insecticidal compounds

Following the isolation procedure previously reported¹²¹ (dry column flash chromatography, flash column chromatography and HPLC) the insecticidal compounds 2 and 3 and a non-insecticidal compound 4 (Figure 2.10, 2.13 and 2.14) were isolated from the combined petroleum ether and ethyl acetate extracts of the stem bark of *Pleurostylia opposita*.

2.3.3 Spectroscopic and structural studies of compound 2

(1) Partial characterisation of compound 2

Compound 2 was a yellow oil and the alkaloidal nature was reconfirmed by using Dragendorff's reagent¹²³. Spectroscopic data including IR, UV, mass and NMR of compound 2 were in good agreement with those reported in a previous investigation¹²¹ of compound 2.1 (Figure 2.5). Spectral data revealed the presence of a β -dihydroagarofuran skeleton with five acetyl and one benzoyl ester groups and a bislactone ring with a 2,3-disubstituted pyridyl skeleton in compound 2 as previously recognised for compound 2.1.

Comparison of the spectral data of compound 2 with those published for compounds containing a β -dihydroagarofuran skeleton (Table 2.6)^{122, 124} suggested the possibility of a 6,6 ring system (Figure 2.7) in 2 rather than a 6,7 ring system as shown in 2.1. To further elucidate the structure of 2, chemical degradation studies were performed. Reduction of the compound 2 was carried

out to cleave the bislactone ring to the corresponding 2,3-disubstituted pyridyl diol and sesquiterpene polyol moiety.

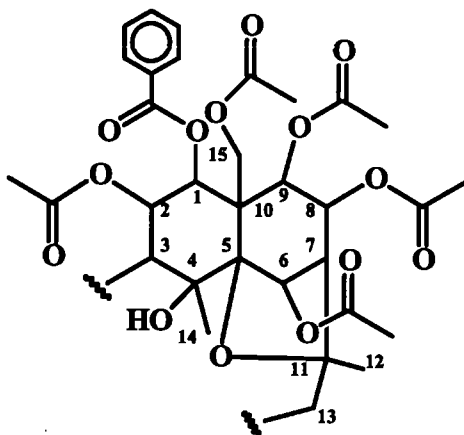


Figure 2.7 : β -dihydroagarofuran ring skeleton of compound 2

Table 2.6 : Comparison of ^{13}C and ^1H NMR spectral data of β -dihydroagarofuran skeleton with those reported for cangorinine **W-II** in CDCl_3

| Position | Reported | Observed | Reported δ_{H} (J) | Observed δ_{H} (J) |
|----------|----------|----------|----------------------------------|----------------------------------|
| 1 | 73.5 | 73.6 | 5.81, d (3.7) | 5.85, d (4.0) |
| 2 | 69.9 | 70.0 | 5.51, dd (3.7, 2.6) | 5.20, dd (4.0, 2.4) |
| 3 | 76.1 | 75.7 | 5.12, d (2.6) | 5.00, d (2.4) |
| 4 | 70.1 | 69.8 | - | - |
| 5 | 93.5 | 94.0 | - | - |
| 6 | 75.3 | 73.6 | 7.08, s | 7.00, s |
| 7 | 51.2 | 50.7 | 2.56, d (3.9) | 2.12, m |
| 8 | 69.3 | 68.8 | 5.60 dd (5.8, 3.9) | 5.55, m |
| 9 | 71.1 | 71.4 | 5.46, d (5.8) | 5.38, d (5.8) |
| 10 | 52.3 | 52.4 | - | - |
| 11 | 84.6 | 84.9 | - | - |
| 12 | 18.1 | 18.0 | 1.71, s | 1.75, s |
| 13 | 70.2 | 69.9 | 5.77, d, 3.71, d (11.8) | 6.00, d, 3.60, d (11.9) |
| 14 | 22.3 | 22.8 | 1.69, d (1.1) | 1.60, s |
| 15 | 60.8 | 60.0 | 5.54, d, 4.49, d (13.3) | 5.34, d, 4.60, d (13.4) |

Chemical shift values are in ppm. Coupling constants (J values) in parentheses are in Hz.

(2) Structural studies of compound 2

(a) Lithium aluminium hydride reduction

Compound 2 was subjected to reduction by lithium aluminium hydride in tetrahydrofuran (THF) under nitrogen for 12 h at room temperature¹²⁵. After work-up, the reaction mixture was filtered and the white solid was washed with ethyl acetate. The filtrate and washings were evaporated to afford, a diol, fragment 1, as a colourless oil (Figure 2.8).

(b) Characterisation of fragment 1

The DEPT and ^{13}C NMR spectra indicated the presence of twelve carbons in fragment 1. The multiplicity studies indicated the presence of one methyl, five methylene, four methine and two quaternary carbons in this fragment. The direct connectivities of carbons and their respective protons were established from the analysis of the ^{13}C - ^1H COSY spectral data (Table 2.7).

Fragment 1 contained a 2,3-disubstituted pyridyl moiety which was identified by comparison of data with compound 2 (Table 2.7 and 2.8). The presence of two primary hydroxyl groups were evident by chemical shifts of carbons which resonated at δ 63.0 and 62.1 in the ^{13}C NMR spectrum of fragment 1.

The characteristic AB type doublet which is associated with protons at δ 4.65 and 4.81 in the ^1H NMR spectrum confirmed that the substituent at 3' of the pyridine ring is $-\text{CH}_2\text{-OH}$. The carbon skeleton at 2' of the pyridine ring was established as in Figure 2.8 by analysis of the ^1H - ^1H -COSY spectral data (Figure 2.9) and the splitting pattern of the protons observed in the ^1H NMR spectrum. The presence of an ethyl substituent at 7' position (Figure 2.8) was confirmed by comparing the ^{13}C chemical shift values of different alkyl chains previously reported¹²⁶. The carbon count of the fragment 1 showed that the methylene group in question is located within the 2' substituent of the pyridine ring (Figure 2.8).

Thus, the structure of the fragment 1 was established as a diol which contains a 2,3-disubstituted pyridyl moiety (Figure 2.8).

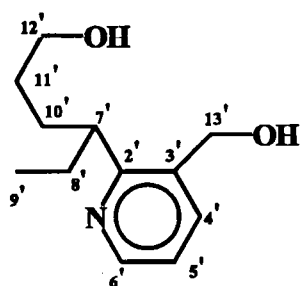


Figure 2.8 : Fragment 1

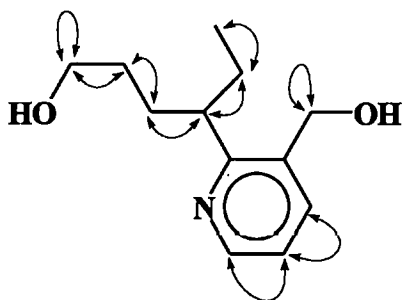


Figure 2.9 : Selected ^1H - ^1H COSY correlations for fragment 1

Table 2.7 : ^{13}C and ^1H NMR assignments of fragment 1 in CDCl_3

| Position | δ_{C} | DEPT | δ_{H} (J) |
|----------|---------------------|-----------------------|-------------------------|
| 2' | 162.8 | C_{q} | - |
| 3' | 134.4 | C_{q} | - |
| 4' | 135.7 | CH | 7.69, dd (7.9, 1.8) |
| 5' | 120.9 | CH | 7.10, dd (7.9, 4.8) |
| 6' | 148.5 | CH | 8.52, d (4.8) |
| 7' | 43.0 | CH | 3.00, m |
| 8' | 28.6 | CH_2 | 2.01, m |
| 9' | 12.1 | CH_3 | 0.74, t (7.3) |
| 10' | 30.2 | CH_2 | 1.92, m |
| 11' | 31.2 | CH_2 | 1.93, m |
| 12' | 63.0 | CH_2 | 3.71, t (5.8) |
| 13' | 62.1 | CH_2 | 4.81, 4.65, d (12.8) |
| 12'-OH | - | - | 2.30-2.50, br s |
| 13'-OH | - | - | 2.30-2.50, br s |

Chemical shift values are in ppm. Coupling constants (J values) in parentheses are in Hz.

(3) Structure of compound 2

On the basis of evidence gathered from chemical and spectral analysis the structure of the compound 2 can be revised as the novel sesquiterpene polyol ester alkaloid containing one benzoyl and five acetyl ester groups. The upper part of the molecule contains a 6,6 ring skeleton based on a β -

dihydroagarofuran core and the lower part a fifteen membered bislactone ring (Figure 2.10).

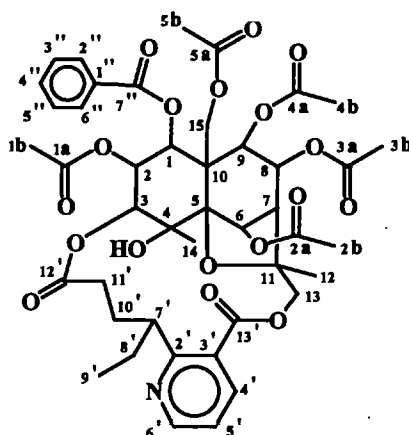


Figure 2.10 : Revised structure for compound 2

Table 2.8 : ^{13}C and ^1H NMR spectral assignments of compound 2 in CDCl_3

| Position | δ_{C} | DEPT | δ_{H} (J) |
|----------|---------------------|---------------|-------------------------|
| 1 | 73.6 | CH | 5.85, d (4.0) |
| 2 | 70.0 | CH | 5.20, dd (4.0, 2.4) |
| 3 | 75.7 | CH | 5.00, d (2.4) |
| 4 | 69.8 | C_q | - |
| 5 | 94.0 | C_q | - |
| 6 | 73.6 | CH | 7.00, s |
| 7 | 50.7 | CH | 2.12, m |
| 8 | 68.8 | CH | 5.55, m |
| 9 | 71.4 | CH | 5.38, d (5.8) |
| 10 | 52.4 | C_q | - |
| 11 | 84.9 | C_q | - |
| 12 | 18.0 | CH_3 | 1.75, s |
| 13 | 69.9 | CH_2 | 6.00, d, 3.60, d (11.9) |
| 14 | 22.8 | CH_3 | 1.60, s |
| 15 | 60.0 | CH_2 | 5.34, d, 4.60, d (13.4) |
| 2' | 168.5 | C_q | - |
| 3' | 129.4 | C_q | - |
| 4' | 138.1 | CH | 8.40, dd (7.9, 1.8) |

| | | | |
|----------|-------|-----------------|---------------------|
| 5' | 120.7 | CH | 7.30, dd (7.9, 4.6) |
| 6' | 153.8 | CH | 8.83, dd (4.6, 1.8) |
| 7' | 42.1 | CH | 4.40, m |
| 8' | 28.8 | CH ₂ | 1.75-2.00, m |
| 9' | 12.0 | CH ₃ | 0.70, t (7.3) |
| 10' | 31.2 | CH ₂ | 2.25-2.35, m |
| 11' | 31.8 | CH ₂ | 1.95-2.20, m |
| 12' | 172.2 | C _q | - |
| 13' | 166.4 | C _q | - |
| 1'' | 125.2 | C _q | - |
| 2'', 6'' | 129.5 | CH | 7.83, d (7.0) |
| 3'', 5'' | 128.5 | CH | 7.42, dd (7.6) |
| 4'' | 133.5 | CH | 7.54, dd (7.6) |
| 7'' | 164.6 | C _q | - |
| 1a | 168.5 | C _q | - |
| 1b | 20.9 | CH ₃ | 2.35, s |
| 2a | 170.0 | C _q | - |
| 2b | 21.0 | CH ₃ | 2.25, s |
| 3a | 169.9 | C _q | - |
| 3b | 21.6 | CH ₃ | 2.30, s |
| 4a | 168.9 | C _q | - |
| 4b | 19.9 | CH ₃ | 1.40, s |
| 5a | 170.4 | C _q | - |
| 5b | 21.4 | CH ₃ | 2.40, s |
| 4-OH | - | - | 4.90, br s |

Chemical shift values are in ppm. Coupling constants (*J* values) in parentheses are in Hz.

The relative stereochemistry of compound **2** was assigned by extensive NOESY experiments (Table 2.9 and Figure 2.11) and the corresponding data are shown in Figure 2.12.

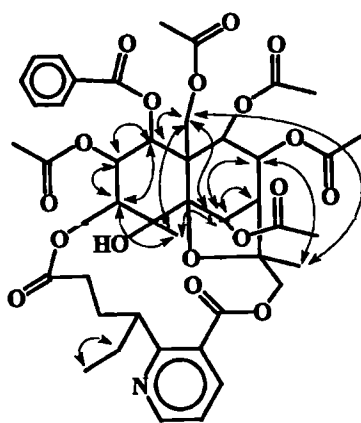


Figure 2.11 : NOESY correlations for compound 2

Table 2.9 : NOESY spectral data for compound 2 in CDCl₃

| Position | δ_H | NOESY (¹ H- ¹ H) |
|----------|--------------|---|
| 1 | 5.85, d | H-2, H-15 |
| 2 | 5.20, dd | H-1, H-3 |
| 3 | 5.00, d | H-1, H-2 |
| 6 | 7.00, s | H-7, H-8, H-15 |
| 7 | 2.12, m | H-6 |
| 8 | 5.55, m | H-6, H-12 |
| 12 | 1.75, s | H-8, H-15 |
| 14 | 1.60, s | H-3, H-6, H-15 |
| 15 | 5.34, d | H-1, H-6, H-8, H-14, H-12 |
| 15 | 4.60, d | H-13, H-14 |
| 8' | 1.95-2.20, m | H-9' |
| 9' | 0.70, t | H-8' |

Chemical shift values are in ppm.

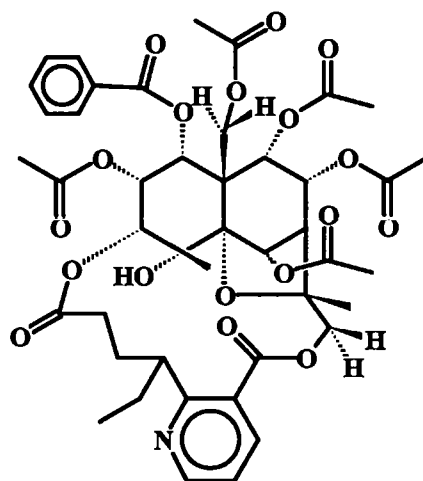


Figure 2.12 : The relative stereochemistry of compound 2

2.3.4 Characterisation of compound 3

Compound 3 was a yellow oil isolated from *Pleurostylia opposita* in 0.02% yield. Spectroscopic data including NOESY correlations of 3 were analogous to those of 2 and suggested the same molecular structure and relative stereochemistry for both compounds. Compound 3 contains six acetyl ester groups while compound 2 has five acetyl and one benzoyl ester groups.

On the basis of spectroscopic evidence the structure of compound 3 was assigned as a fifteen membered macrocyclic sesquiterpene polyol ester alkaloid (Figure 2.13).

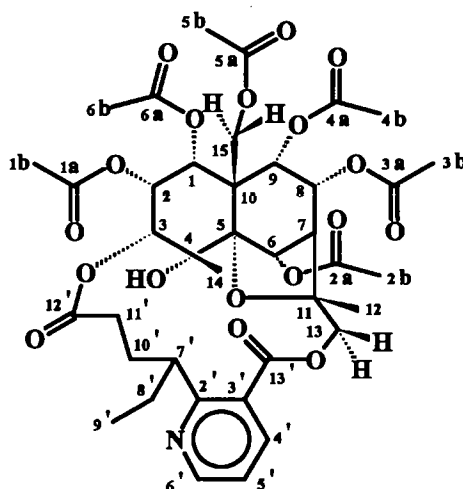


Figure 2.13 : Compound 3

Table 2.10 : ^{13}C and ^1H NMR assignments for compound 3 in CDCl_3

| Position | δ_{C} | DEPT | δ_{H} (J) |
|----------|---------------------|------|-------------------------|
| 1 | 73.4 | CH | 5.55, d (3.9) |
| 2 | 69.7 | CH | 5.20, dd (3.9, 2.4) |
| 3 | 75.7 | CH | 4.97, d (2.4) |

| | | | |
|------|-------|-----------------|-------------------------|
| 4 | 69.6 | C _q | - |
| 5 | 93.9 | C _q | - |
| 6 | 73.6 | CH | 7.00, s |
| 7 | 50.8 | CH | 2.12, m |
| 8 | 68.9 | CH | 5.55, m |
| 9 | 70.8 | CH | 5.48, d (5.8) |
| 10 | 52.1 | C _q | - |
| 11 | 84.7 | C _q | - |
| 12 | 18.0 | CH ₃ | 1.73, s |
| 13 | 69.9 | CH ₂ | 6.00, d, 3.60, d (11.7) |
| 14 | 22.9 | CH ₃ | 1.68, s |
| 15 | 60.0 | CH ₂ | 5.15, d, 4.50, d (13.5) |
| 2' | 168.0 | C _q | - |
| 3' | 129.6 | C _q | - |
| 4' | 138.2 | CH | 8.40, dd (7.9, 1.8) |
| 5' | 120.8 | CH | 7.13, dd (7.9, 4.6) |
| 6' | 153.8 | CH | 8.83, dd (4.6, 1.8) |
| 7' | 42.2 | CH | 4.40, m |
| 8' | 31.8 | CH ₂ | 1.95-2.20, m |
| 9' | 28.8 | CH ₂ | 1.75-2.00, m |
| 10' | 31.3 | CH ₂ | 2.25-2.35, m |
| 11' | 31.8 | CH ₂ | 1.95-2.20, m |
| 12' | 172.3 | C _q | - |
| 12' | 166.4 | C _q | - |
| 1a | 168.0 | C _q | - |
| 1b | 21.0 | CH ₃ | 2.15, s |
| 2a | 169.9 | C _q | - |
| 2b | 20.8 | CH ₃ | 2.15, s |
| 3a | 170.1 | C _q | - |
| 3b | 21.6 | CH ₃ | 2.20, s |
| 4a | 168.8 | C _q | - |
| 4b | 20.5 | CH ₃ | 1.30, s |
| 5a | 170.3 | C _q | - |
| 5b | 21.3 | CH ₃ | 2.35, s |
| 6a | 169.1 | C _q | - |
| 6b | 25.3 | CH ₃ | 1.90, s |
| 4-OH | - | - | 4.90, br s |

Chemical shift values are in ppm. Coupling constants (*J* values) in parentheses are in Hz.

2.3.5 Characterisation of compound 4

Compound 4 was a yellow oil, isolated from *Pleurostylia opposita* in 0.01% yield. Comparison of the NMR spectral data of 4 with those of 2 (Table 2.8 and 2.11) revealed the close structural similarity of the two compounds and the presence of four acetyl, one benzoyl and one nicotinyl ester groups in compound 4. In addition, NOESY spectral data of 4 was consistent with those of 2, establishing the same relative stereochemistry for both compounds (Figure 2.14). Thus, the structure of 4 was established as a sesquiterpene polyol ester alkaloid which contains a fifteen membered bislactone ring (Figure 2.14).

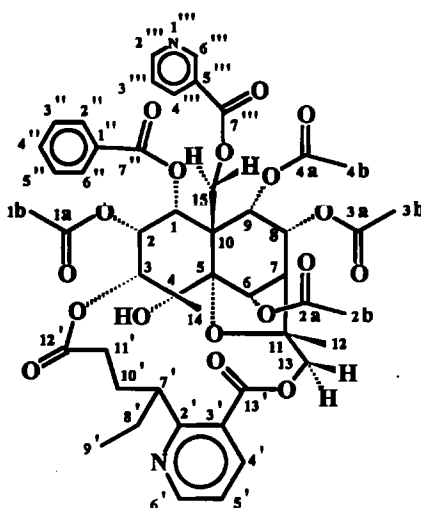


Figure 2.14 : Compound 4

Table 2.11 : ^{13}C and ^1H NMR assignments for compound 4 in CDCl_3

| Position | δ_{C} | DEPT | δ_{H} (J) |
|----------|---------------------|-----------------------|-------------------------|
| 1 | 73.9 | CH | 6.05, d (4.0) |
| 2 | 71.2 | CH | 5.20, dd (4.0, 2.4) |
| 3 | 75.5 | CH | 5.15, d (2.4) |
| 4 | 69.9 | C_{q} | - |
| 5 | 94.0 | C_{q} | - |

| | | | |
|----------|-------|-----------------|-------------------------|
| 6 | 73.6 | CH | 7.06, s |
| 7 | 50.8 | CH | 2.12, m |
| 8 | 68.9 | CH | 5.55, m |
| 9 | 71.2 | CH | 5.65, d (5.8) |
| 10 | 52.4 | C _q | - |
| 11 | 85.1 | C _q | - |
| 12 | 18.1 | CH ₃ | 1.75, s |
| 13 | 70.3 | CH ₂ | 6.05, d, 3.40, d (11.9) |
| 14 | 23.2 | CH ₃ | 1.65, s |
| 15 | 60.3 | CH ₂ | 5.45, d, 4.55, d (13.4) |
| 2' | 167.9 | C _q | - |
| 3' | 128.6 | C _q | - |
| 4' | 137.6 | CH | 8.50, dd (7.9, 1.8) |
| 5' | 121.0 | CH | 7.40, dd (7.9, 4.6) |
| 6' | 153.9 | CH | 8.90, dd (4.6, 1.8) |
| 7' | 42.2 | CH | 4.40, m |
| 8' | 28.7 | CH ₂ | 1.75-2.00, m |
| 9' | 12.0 | CH ₃ | 0.72, t (7.3) |
| 10' | 31.2 | CH ₂ | 2.25-2.35, m |
| 11' | 31.8 | CH ₂ | 1.95-2.20, m |
| 12' | 172.2 | C _q | - |
| 13' | 166.1 | C _q | - |
| 1'' | 124.9 | C _q | - |
| 2'', 6'' | 129.5 | CH | 7.75, d (7.0) |
| 3'', 5'' | 128.6 | CH | 7.30, dd (7.6) |
| 4'' | 133.6 | CH | 7.54, dd (7.6) |
| 7'' | 164.6 | C _q | - |
| 2''' | 153.5 | CH | 8.90, br s |
| 3''' | 124.8 | CH | 7.50, m |
| 4''' | 138.6 | CH | 8.40, m |
| 5''' | 123.8 | C _q | - |
| 6''' | 150.8 | CH | 9.30, br s |
| 7''' | 163.4 | C _q | - |
| 1a | 168.9 | C _q | - |
| 1b | 21.3 | CH ₃ | 2.34, s |
| 2a | 170.0 | C _q | - |
| 2b | 20.9 | CH ₃ | 2.14, s |
| 3a | 170.0 | C _q | - |
| 3b | 21.7 | CH ₃ | 2.23, s |
| 4a | 168.4 | C _q | - |
| 4b | 19.9 | CH ₃ | 1.35, s |
| 4-OH | - | - | 4.90, br s |

Chemical shift values are in ppm. Coupling constants (*J* values) in parentheses are in Hz.

Compounds 2, 3 and 4 are analogues of the known sesquiterpene polyol ester alkaloids. They all possess a 6,6 β -dihydroagarofuran ring skeleton and a fifteen membered bislactone ring containing a 2,3-disubstituted pyridine moiety. The carbon skeleton of compounds 2, 3 and 4 has a close resemblance to that of cassinin¹²⁷ but with different ester functionalities. The esterification pattern found in compounds 2-4 is unique among the reported sesquiterpene polyol ester alkaloids within the family Celastraceae.

2.4 ISOLATION AND CHARACTERISATION OF INSECTICIDAL COMPOUNDS FROM *ALSEODAPHNE SEMICARPIFOLIA*

2.4.1 Introduction

Alseodaphne semicarpifolia (Lauraceae) is a large timber tree, growing in the central province of Sri Lanka. A few phytochemical constituents (Figure 2.15) including alkaloids¹²⁸ and a phenanthrenoid (perakensol)¹²⁹ have been reported from the genus *Alseodaphne* but none of them are linked to any biological activity. Phytochemical studies of *A. semicarpifolia* have not been reported previously.

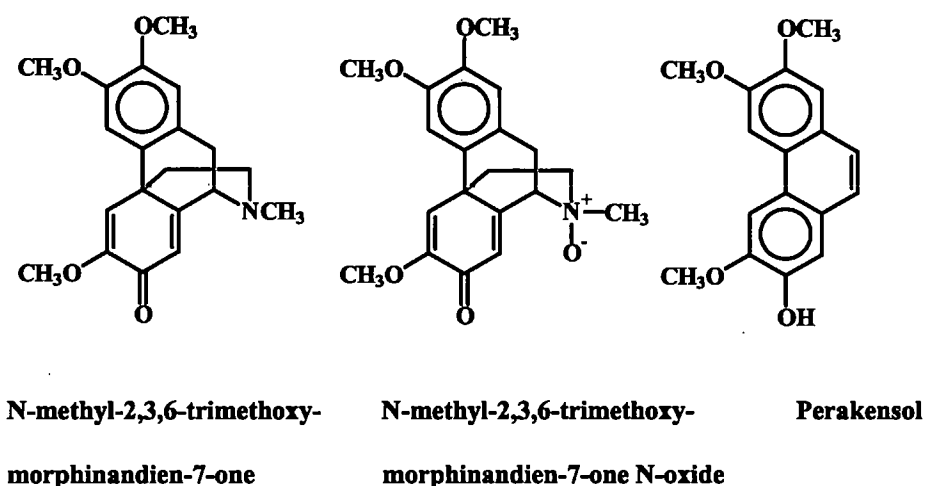


Figure 2.15 : Alkaloids and phenanthrenoid

Preliminary bio-assay studies of the insecticidal activity of the petroleum ether, ethyl acetate and ethanol extracts of the stem bark of this plant showed moderate insecticidal activity against houseflies (Table 2.12).

Table 2.12 : Insecticidal activity of *Alseodaphne semicarpifolia*

| Insect species | Stem bark | | |
|-----------------|-----------|----|----|
| | A | B | C |
| MB [*] | WT | WT | WT |
| HF [*] | 89 | NT | NT |
| PX [†] | NT | NT | NT |
| BT [‡] | NT | NT | NT |
| TU [¶] | NT | NT | NT |

Insecticidal activity is given as % mortality (* 20 µg/insect, † 10 µg/insect, ‡ 2000 ppm, ¶ 1000 ppm).

NT: non toxic, WT : weakly toxic (<20%). MB : mustard beetle (*Phaedon cochleariae*),

HF: housefly (*Musca domestica*), PX : larvae of diamondback moth (*Plutella xylostella*),

BT : whitefly (*Bemisia tabaci*), TU : mites (*Tetranychus urticae*).

A : petroleum ether, B : ethyl acetate, C : ethanol

2.4.2 Isolation of insecticidal compound

Sequential extraction of the stem bark of *A. semicarpifolia* showed that the petroleum ether extract contains the components responsible for activity. Dry column flash chromatography of this extract using a mixture of petroleum ether and diethyl ether gave a insecticidal compound **5** (Figure 2.17) as a white crystalline solid which showed toxicity against houseflies.

2.4.3 Characterisation of compound **5**

Compound **5** was isolated as a white crystalline solid (m.p. 123.5 °C). The Electron Impact (EI) mass spectrum displayed the molecular ion at m/z 354 which corresponds to a molecular formula of $C_{20}H_{18}O_6$. The presence of a methylenedioxyphenyl group was evident from the strong band at 1245 cm^{-1} in

the IR spectrum. It also showed the absorptions characteristic for a phenyl ring (1609 cm^{-1}).

The ^{13}C NMR spectrum indicated the presence of ten carbons in the molecule, of which four were sp^3 and the rest were sp^2 hybridised. The multiplicities of these carbons were determined by DEPT experiment as two methylenes, five methines and three quaternary carbons (Table 2.13). The ^{13}C NMR spectrum showed a peak at δ 101.0 suggesting the possible presence of an anomeric carbon in the molecule. In addition, four oxygenated carbons were observed at δ 71.6, 85.7, 147.0 and 147.9 in the ^{13}C NMR spectrum. The ^1H NMR spectrum of **5** exhibited the presence of methylenedioxy protons at δ 5.94 (s), a bridge head proton at δ 3.04 (br s) and aromatic protons at δ 6.79 and 6.84. It also showed the presence of one benzylic proton at δ 4.70 and methyleneoxy protons at δ 3.85 and 4.22 in the molecule.

On the basis of NMR and mass spectral data, compound **5** was identified as a sesamin type lignan having two identical aromatic rings with a $-\text{OCH}_2\text{O}-$ substituent (Figure 2.16).

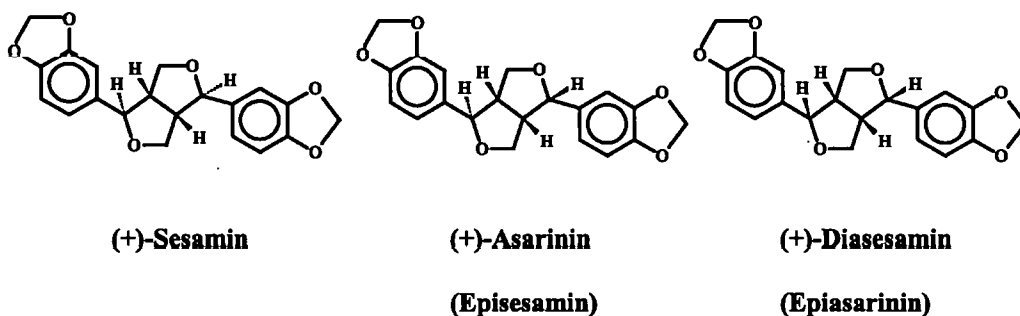


Figure 2.16 : Stereoisomers of the sesamin series

Three stereoisomers (+)-sesamin, (+)-asarinin (episesamin) and (+)-diasesamin (epiasarinin) (Figure 2.16) are possible structures for satisfying the molecular formula $C_{20}H_{18}O_6$. The symmetry of the molecule was suggested by the NMR spectral data (Table 2.13). The structure of **5** was established as (+)-sesamin (Figure 2.17) based on the α_D value (+64.5) and the coupling constants of the hydrogens at 2 and 3 positions which were in agreement with those reported for (+)-sesamin¹³⁰. The NMR spectral data of **5** are in agreement with those previously reported for (+)-sesamin (Table 2.13)¹³¹.

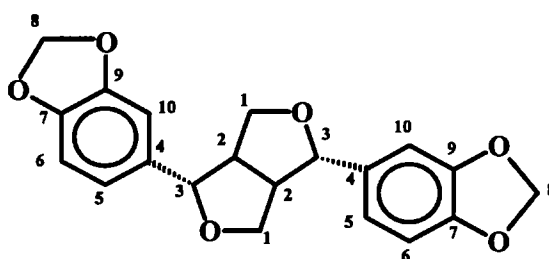


Figure 2.17 : Compound **5**

Table 2.13 : ^{13}C and 1H NMR assignments for compound **5** in $CDCl_3$

| Position | Observed δ_C | Reported δ_C | DEPT | Observed δ_H (J) |
|----------|---------------------|---------------------|-----------------|---|
| 1 | 71.6 | 71.5 | CH ₂ | 4.22, dd (9.2, 6.7), 3.85, dd (9.2, 3.6) |
| 2 | 54.2 | 54.2 | CH | 3.04, br s |
| 3 | 85.7 | 85.6 | CH | 4.70, d (4.2) |
| 4 | 134.9 | 134.9 | C _q | - |
| 5 | 108.1 | 107.9 | CH | 6.79, m |
| 6 | 106.4 | 106.3 | CH | 6.79, m |
| 7 | 147.0 | 146.8 | C _q | - |
| 8 | 101.0 | 100.9 | CH ₂ | 5.94, s |
| 9 | 147.9 | 147.7 | C _q | - |
| 10 | 119.3 | 119.1 | CH | 6.84, d (0.9) |

Chemical shift values are in ppm. Coupling constants (J values) in parentheses are in Hz.

The selected ^1H - ^1H COSY correlations of **5** are illustrated in Figure 2.18. This is the first report of the isolation of (+)-sesamin from *Alseodaphne semicarpifolia*.

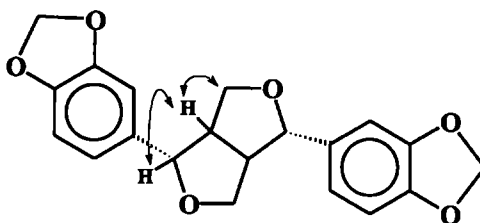


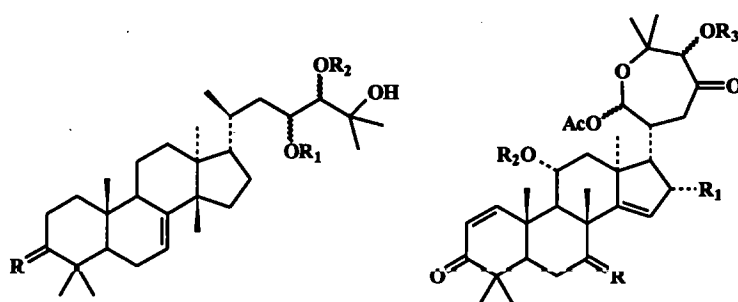
Figure 2.18 : Selected ^1H - ^1H COSY correlations for compound **5**

(+)-Sesamin has been previously isolated from the bark of various *Fagara* species and it is a minor constituent of the seed oil of *Sesamum indicum*, commonly known as sesame oil¹³². (+)-Sesamin is known to be a synergist with pyrethrins against houseflies and its activity is due to the methylenedioxyphenyl group¹³².

2.5 ISOLATION AND CHARACTERISATION OF INSECTICIDAL COMPOUNDS FROM *WALSURA PISCIDIA*

2.5.1 Introduction

Walsura piscidia Roxb., (Meliaceae) is a large timber tree, widely distributed in the dry zone of Sri Lanka. Various parts of this plant are used in traditional medicine in Asia. Although reports related to the insecticidal activity of this plant were not found, a series of tirucallanes (piscidinols) and tetranortriterpenoids including azalone has been isolated, and their chemistry has been established (Figure 2.19)¹³³.



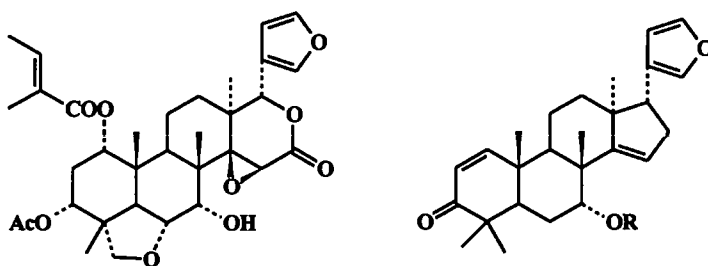
Piscidinol A R = O; R₁ = R₂ = H

Piscidinol C R = H, β-OH; R₁ = R₂ = R₃ = H

Piscidinol B R = H, β-OH; R₁ = R₂ = H

Piscidinol D R = H, α-OH; R₁ = OH; R₂ = R₃ = H

Piscidinol E R = O; R₁ = OH; R₂ = R₃ = H



Piscidofuran

Azalone = 7-deacetoxy-7-hydroxyazadirone R = H

1,2-dihydro derivative R = H; 1,2 - dihydro

Figure 2.19 : Piscidinols and tetranortriterpenoids

Preliminary bio-assay studies of the petroleum ether extract of the stem bark showed moderate toxicity against mustard beetles but was non-toxic to housefly (Table 2.14).

Table 2.14 : Insecticidal activity of *Walsura piscidia*

| Insect species | Stem bark | | |
|-----------------|-----------|----|----|
| | A | B | C |
| MB [*] | 100 | NT | NT |
| HF [*] | NT | NT | NT |
| PX [†] | NT | NT | NT |
| BT [‡] | NT | NT | NT |
| TU [¶] | NT | NT | NT |

Insecticidal activity is given as % mortality (^{*}20 µg/insect, [†]10 µg/insect, [‡]2000 ppm, [¶]1000 ppm).

NT: non toxic. MB : mustard beetle (*Phaedon cochleariae*), HF: housefly (*Musca domestica*),

PX : larvae of diamondback moth (*Plutella xylostella*), BT : whitefly (*Bemisia tabaci*),

TU : mites (*Tetranychus urticae*). A: petroleum ether, B : ethyl acetate, C : ethanol

2.5.2 Isolation of Insecticidal compound

The active petroleum ether extract of the stem bark of *Walsura piscidia* was subjected to dry column flash chromatography and further purification of the major fraction allowed the isolation of compound 6 (Figure 2.20) which showed insecticidal activity.

2.5.3 Characterisation of compound 6

Compound 6 was a white crystalline solid (m.p. 194-195 °C) isolated from *Walsura piscidia* in 0.014% yield. The Electron Impact (EI) mass spectrum showed the molecular ion at m/z 394, suggesting the molecular formula to be $C_{26}H_{34}O_3$. Compound 6 showed IR absorptions characteristic of an α , β -unsaturated ketone (1704 cm^{-1}) and hydroxyl (3587 cm^{-1}) functionalities. The UV spectrum ($\lambda_{\text{max}} = 216\text{ nm}$) also suggested the presence of an α , β -unsaturated ketone.

The ^{13}C NMR spectrum showed the presence of twenty six carbons in the molecule which were identified as five methyl, four methylene, ten methine and seven quaternary carbons by DEPT spectral data. The ^1H NMR spectrum showed the presence of thirty four protons in the molecule of which six are olefinic. The presence of an α , β -unsaturated double bond was evident by the characteristic chemical shifts in the ^{13}C and ^1H NMR spectral data (δ_{C} 158.2 and 125.4; δ_{H} 7.14, d, $J = 10.3\text{ Hz}$ and 5.80, d, $J = 10.3\text{ Hz}$).

The C-H connectivities of the molecule were assigned unambiguously by analysing ^{13}C - ^1H COSY spectral data. The presence of a furan ring in the molecule was evident by the characteristic ^{13}C and ^1H NMR chemical shifts which resonated at δ_{C} 125.4, 139.2, 124.0, 142.6 and δ_{H} 7.38, 7.27, 5.84, respectively.

Table 2.15 : ^{13}C and ^1H NMR data for compound 6 and reported NMR data in

CDCl_3

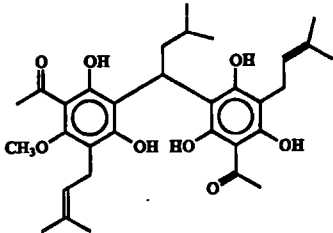
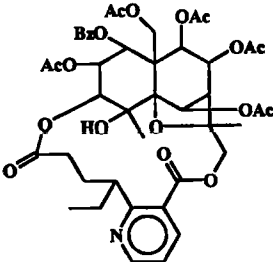
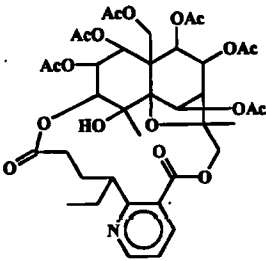
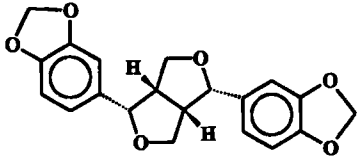
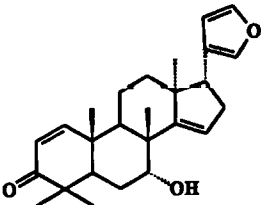
| Position | δ_{C} | DEPT | δ_{H} (J) |
|----------|---------------------|---------------|-------------------------|
| 1 | 158.2 | CH | 7.14 d, (10.3) |
| 2 | 125.4 | CH | 5.84, d, (10.3) |
| 3 | 205.1 | C_q | - |
| 4 | 40.2 | C_q | - |
| 5 | 44.8 | CH | 2.41, m |
| 6 | 24.6 | CH_2 | 1.88, 1.89, m |
| 7 | 71.5 | CH | 4.17, d (7.3) |
| 8 | 44.4 | C_q | - |
| 9 | 44.2 | CH | 2.41, m |
| 10 | 47.3 | C_q | - |
| 11 | 16.2 | CH_2 | 1.18-1.21, m |
| 12 | 32.6 | CH_2 | 1.70-1.89, m |
| 13 | 47.3 | C_q | - |
| 14 | 161.2 | C_q | - |
| 15 | 111.0 | CH | 6.29, s |
| 16 | 34.3 | CH_2 | 2.41-2.60, m |
| 17 | 51.5 | CH | 2.86, dd (7.3, 7.2) |
| 18 | 124.0 | C_q | - |
| 19 | 125.4 | CH | 7.38, d (1.8) |
| 20 | 139.2 | CH | 7.27, s |
| 21 | 142.6 | CH | 5.84, d (10.0) |
| 22 | 27.5 | CH_3 | 1.18, s |
| 23 | 27.1 | CH_3 | 1.18, s |
| 24 | 18.9 | CH_3 | 1.16, s |
| 25 | 20.2 | CH_3 | 0.80, s |
| 26 | 21.5 | CH_3 | 1.10, s |

Chemical shift values are in ppm. Coupling constants (J values) in parentheses are in Hz.

2.6 INSECTICIDAL ACTIVITY

The five insecticidal compounds (1-6) isolated from the extracts of four plant species, *Acronychia pedunculata*, *Pleurostylia opposita*, *Alseodaphne semicarpifolia* and *Walsura piscida* showed significant activity against

Table 2.16 : Insecticidal activity of natural products 1-6

| Compound | MB | HF | BT | PX |
|---|-------|------|----|----|
|  | 0.8* | 2.2* | NT | NT |
|  | 0.02* | NT | NT | NT |
|  | 0.1* | NT | NT | NT |
|  | WT | 87** | NT | NT |
|  | 100** | NT | NT | NT |

* Insecticidal activity is given in I.D₅₀ (μg/insect) or as ** % mortality (20 μg/insect)

NT: non toxic, WT : weakly toxic (<20%). MB : mustard beetle (*Phaedon cochleariae*),

HF: housefly (*Musca domestica*) PX : diamondback moth larvae (*Plutella xylostella*),

BT: whitefly (*Bemisia tabaci*).

mustard beetles and houseflies (Table 2.16). Compound 1 was active against mustard beetles and houseflies, while 2, 3 and 5 were active against only mustard beetles and 4 was active against only houseflies. None of the compounds displayed activity against whiteflies and larvae of diamondback moth.

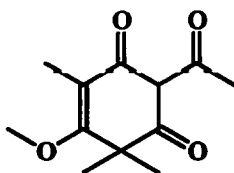
2.7 SYNTHESIS STUDIES OF ACROVESTONE AND ANALOGUES

2.7.1 Introduction

Acrovestone (1), the insecticidal natural product isolated from *A. pedunculata* (Rutaceae) was selected for synthetic studies due to its promising insecticidal activity identified in this study and previously reported cytotoxic activity. It was envisaged that SAR studies based on this compound would be interesting since the molecule has a dense array of functional groups and a relatively fixed geometry. A synthesis of acrovestone was designed using the postulated biosynthetic route which is described in the following section.

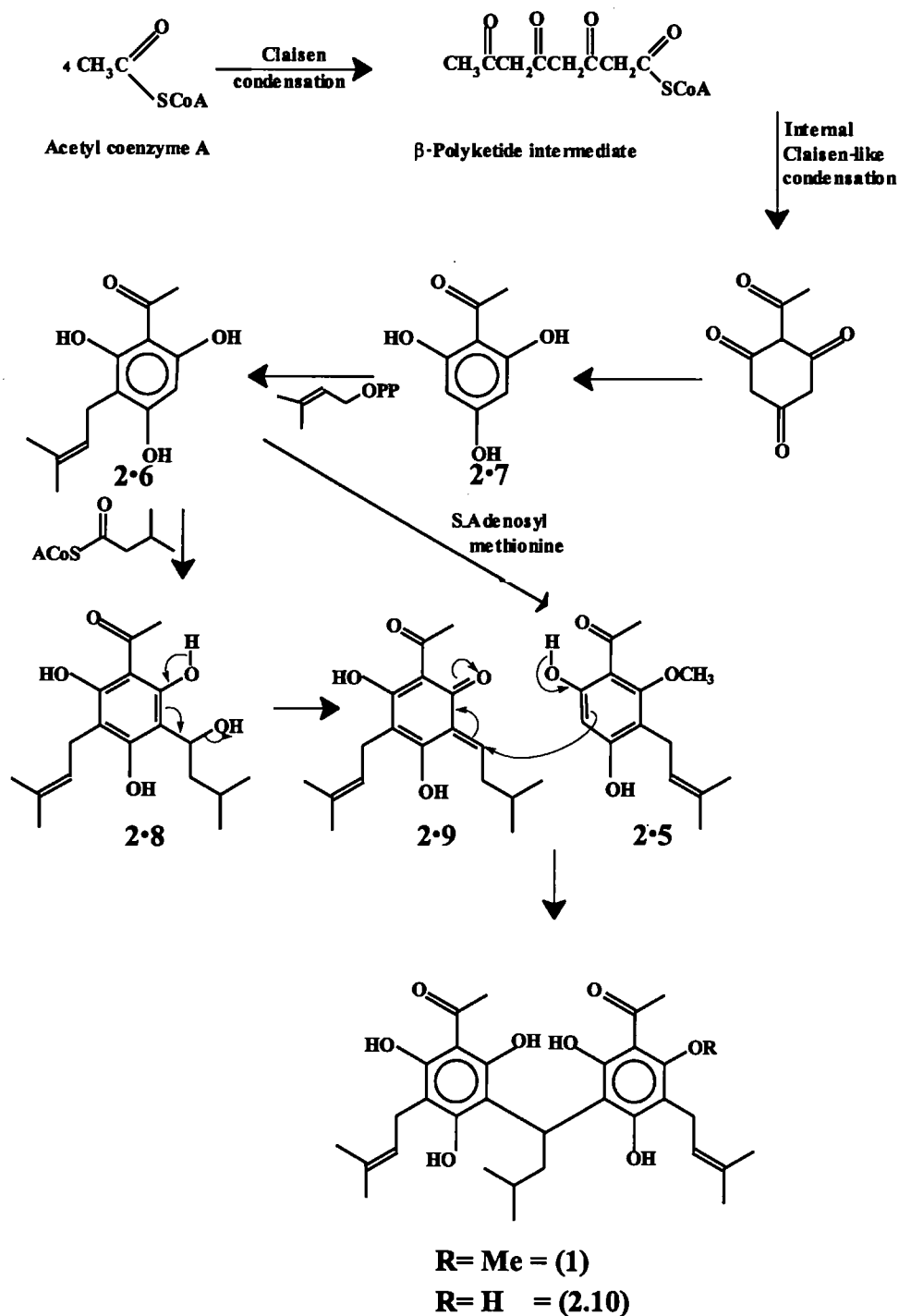
2.7.2 The postulated biosynthesis of acrovestone

Reports on biosynthetic studies of phloroglucinol derivatives have been limited to hop bitter acids¹³⁶ and tasmanone (2.4)¹³⁷ (Figure 2.22). The biosynthesis of acrovestone can be postulated by analogy with the biosynthesis of these compounds (Scheme 2.1).



2.4

Figure 2.22 : Tasmanone



Scheme 2.1 : Postulated biosynthesis of acrovestone

Biosynthetically acrovestone could be derived by condensation of two aryl ketone precursors, acronylin (2.5) and demethylacronylin (2.6) with an

isovaleryl C-4 unit. The first step in the biosynthesis could be the formation of 2,4,6-trihydroxyacetophenone (2.7) *via* the polyketide pathway (Scheme 2.1)¹³⁸. Nuclear prenylation of 2,4,6-trihydroxyacetophenone will result in demethylacronylin (2.6) and selective O-methylation may occur at some stage resulting in acronylin (2.5). Previous studies on phloroglucinol compounds indicated that the biological precursor for the methyl group of O-methylation is S-Adenosyl methionine¹³⁷.

The insertion of an isovaleryl group to demethylacronylin (2.6) could yield the intermediate 2.8. The route to acrovestone (1) may involve the intermediacy of the *O*-quinone methide (2.9), formally derivable from intermediate 2.8. This reacts in an aromatic electrophilic substitution at the activated carbon of acronylin (2.5) leading to acrovestone. Similarly, electrophilic substitution has to occur at the activated carbon of demethylacronylin (2.6) to yield demethylacrovestone (2.10).

2.7.3 Synthesis of acrovestone

A large number of bio-active acyl phloroglucinols have been reported from the family Myrtaceae, especially from *Eucalyptus* species¹³⁹. Recent studies have paid more attention to robustadials¹⁴⁰ and several macrocarpals¹⁴¹ because of their pharmacological activity. However, to date, there are no reports on the biosynthesis and synthesis of acrovestone.

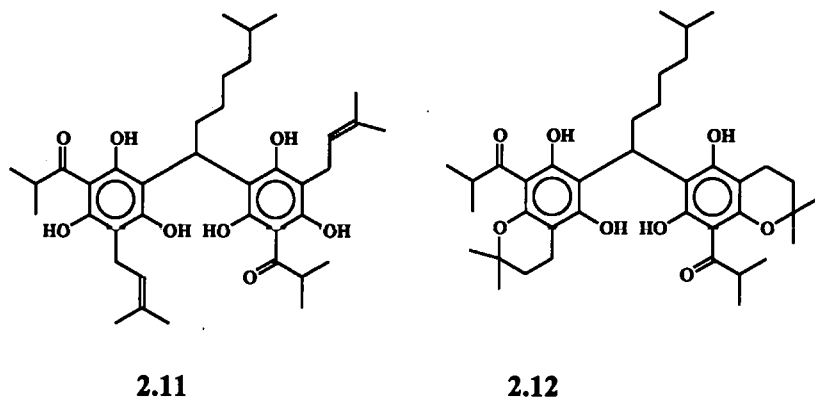
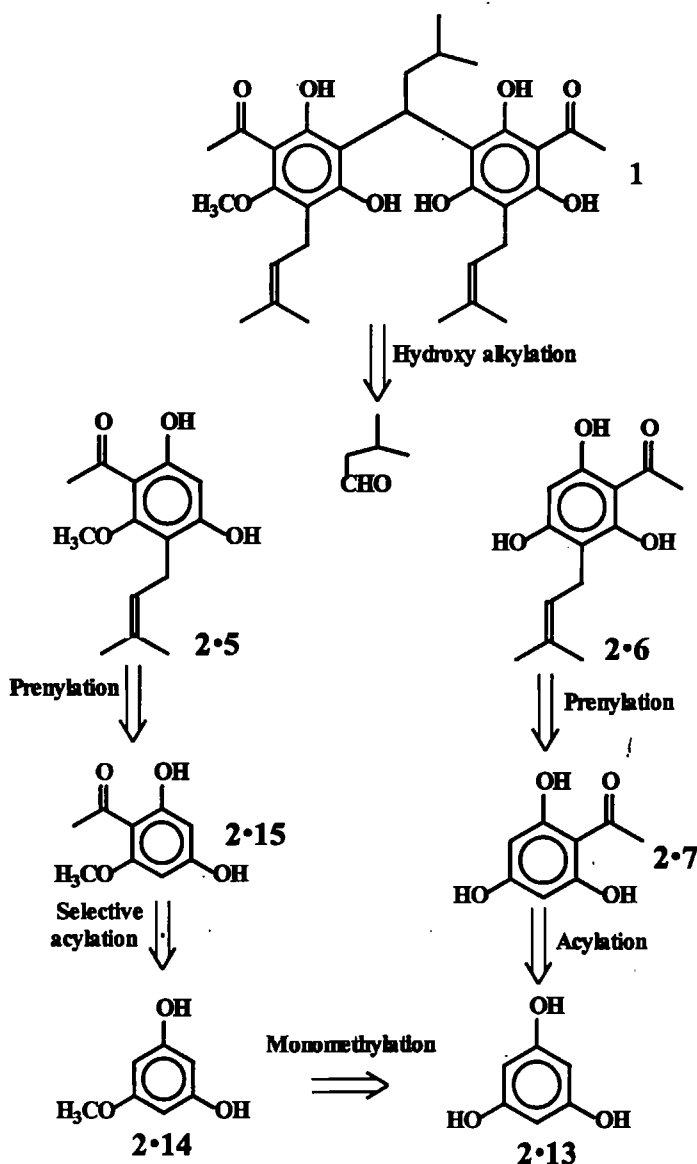


Figure 2.23 : Phloroglucinol derivatives from *Helichrysum platyerum*

Acrovestone (1) and demethylacrovestone (2.10) have previously only been isolated from the genus *Acronychia*, but the structurally similar aryl ketone dimer 2.11 has been isolated from *Helichrysum platyerum* (Compositae) (Figure 2.23)¹⁴². Attempts to synthesise 2.11 resulted only in the corresponding chroman 2.12 (Figure 2.23)¹⁴³.



Scheme 2.2 : Retrosynthetic analysis of acrovestone/demethylacrovestone

In the present study, the synthesis of acrovestone (1) and demethylacrovestone (2.10) from commercially available phloroglucinol was designed according to the retrosynthetic analysis depicted in Scheme 2.2 in which hydroxy alkylation and nuclear prenylation¹⁴⁴ are the two key reactions. Acylation of phloroglucinol with subsequent C-prenylation should result in

demethylacronylin (2.6). Similarly, regioselective acylation of monomethylated phloroglucinol followed by C-prenylation should result in acronylin (2.5). Finally, condensation of 2.5 and 2.6 with isovaleraldehyde in the presence of a suitable catalyst should lead to the formation of acrovestone (1) and demethylacrovestone (2.10), respectively. The following sections discuss the practical aspects of the synthesis of acrovestone, demethylacrovestone and their analogues.

2.7.4 Synthesis of demethylacronylin

Demethylacronylin, 2,4,6-trihydroxy-3-isopentenylacetophenone (2.6) has been isolated previously from the root bark of *A. pedunculata*¹⁰⁷ and the synthesis has been reported in poor yields^{145, 146}.

To synthesise 2.6 in higher yields, the previously reported C-alkylation methods were examined in the present study. C-alkylation of phenols is traditionally carried out in acidic or basic media¹⁴⁴. Unusual Friedel-Crafts methodologies such as those utilising aluminium oxide¹⁴⁷, platinum catalysis¹⁴⁸, and silicon chemistry¹⁴⁹ have been developed recently for this purpose. However, these methods have not been used for the synthesis of demethylacronylin (2.6) or acronylin (2.5) previously. In the present study, the synthesis of 2.6 was attempted by modifications to the already reported unusual Friedel-Craft methodologies.

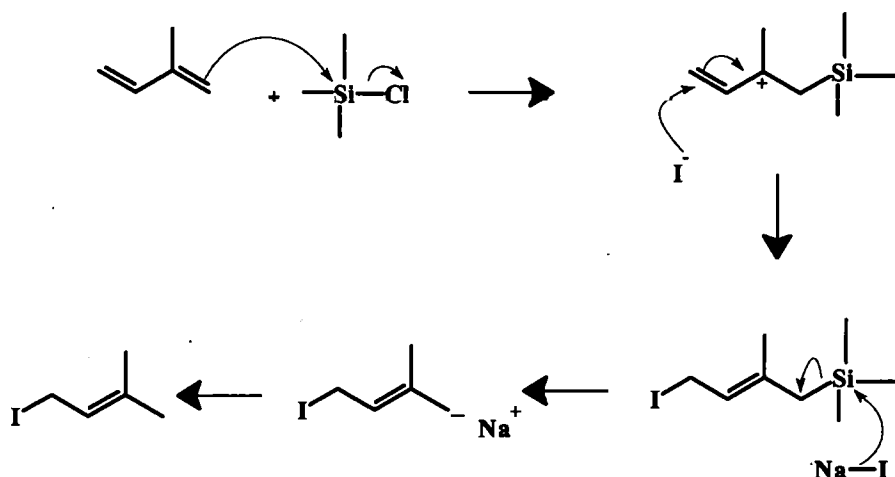
(a) Friedel-Craft alkylation with Lewis acid

The use of a Lewis acids, in particular boron trifluoride-etherate ($\text{BF}_3\text{-Et}_2\text{O}$), for nuclear prenylation has been previously described¹⁵⁰. But the reported yields for the synthesis of 2.6 using $\text{BF}_3\text{-Et}_2\text{O}$ were poor. The same procedure was used for the current synthesis and the required 2.6 was obtained in 12% yield with many polyalkylated byproducts. Simple modifications of the reaction conditions such as temperature and time did not improve the yield.

(b) Aluminium oxide promoted C-alkylation

Aluminium oxide promoted C-alkylation has been reported for a number of phenolic compounds¹⁴⁷. Although this methodology has shown wide applicability and often high yields, examples have not been found for prenylation of phloroglucinols or polyhydroxyacetophenones. In this study aluminium oxide promoted synthesis of demethylacronylin (2.6) gave only chroman 2.16 (In page 93), the cyclic derivative of 2.6. This indicated that aluminium oxide cannot be used for the synthesis of 2.6 in good yields.

(c) Silicon mediated C-alkylation



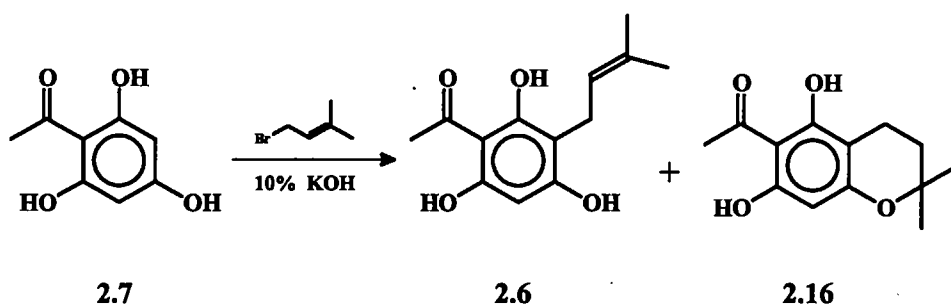
Scheme 2.3 : Mechanism for the *in situ* generation of prenyl cation

Silicon mediated C-alkylations have been reported for the synthesis of prenylated naphthalenes and phenols¹⁴⁹. In this method, the isopentenyl synthon has been generated *in situ* as rationalised in Scheme 2.3. In the present study, demethylacronylin (2.6) was achieved only in 12% and chroman (2.16) in 35% yields using a similar procedure described in literature¹⁴⁹.

(d) Base catalysed C-alkylation

The target compound 2.6 has previously been synthesised by base catalysed C-alkylation reaction¹⁴⁵. The main problems associated with this methodology were poly C-alkylation, O-alkylation, cyclisation and deacylation. Initial experiments based on base catalysed C-alkylation reaction¹⁴⁵ showed the potential for further improvements, therefore the reaction conditions were

investigated in detail. It was envisaged that use of alcoholic or aqueous potassium hydroxide (KOH) (10%) has either little or no effect on the formation of compound 2.6. Demethylacronylin (2.6) and chroman 2.16 were obtained in 12% and 35% yields, respectively, with 10% KOH at room temperature under N₂ (Table 2.17 and Scheme 2.4).



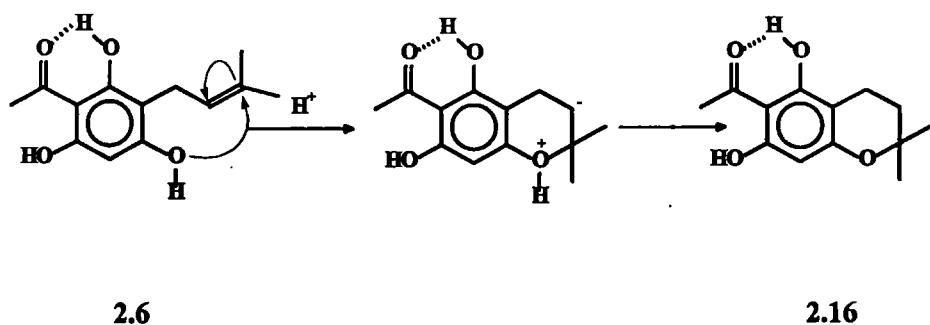
Scheme 2.4 : Aqueous KOH promoted C-alkylation

Table 2.17. : Optimisation of conditions for base catalysed prenylation

| Entry | Condition ^b | | Yield ^a (%) | |
|-------|------------------------|-----------------------------|------------------------|------|
| | Temp °C | Solvent | 2.6 | 2.16 |
| 1 | -10 | 10% aq KOH | ca. 3 | - |
| 2 | rt | 10% aq KOH | 12 | 35 |
| 3 | 0 | 10% aq KOH | 15 | 20 |
| 4 | 0 | 10% aq KOH degassed for 3 h | 55 | 15 |
| 5 | 0 | 10% MeOH-KOH | 15 | 20 |

^a Yields were based on isolated products. ^b Reaction under N₂

The cyclisation of **2.6** can be rationalised as in Scheme 2.5. These results indicated that cyclisation can be promoted either by acid or temperature (Table 2.17).



Scheme 2.5 : Chroman formation

Having identified the two main factors which influence the cyclisation, the reaction was carried out at -10°C and obtained a 2-3% yield of desired **2.6** but not chroman **2.16** (Table 2.17). Freshly prepared aqueous 10% KOH solution was vigorously degassed over 3 h and the reaction was carried out under a flow of N_2 at 0°C for 2 h to afford **2.6** and **2.16** in 55% and 15% yields, respectively (Table 2.17).

The separation of demethylacronylin (**2.6**) from the starting material, (2,4,6-trihydroxyacetophenone) was difficult by chromatography. The crude reaction mixture was dissolved in dichloromethane which allowed the separation of crude demethylacronylin (**2.6**) as a yellow solid. Demethylacronylin (**2.6**) was purified from the unreacted starting material, 2,4,6-trihydroxyacetophenone by

recrystallisation with diethyl ether:petroleum ether (9:1, v/v) as a pale yellow crystalline solid.

2.7.5 Synthesis of acronylin

Acronylin (2.5) has been isolated¹⁰⁸ previously from the stem bark of *A. pedunculata* and a non-regioselective synthesis has been reported¹⁵¹. This method was adapted for the present study. Friedel-Crafts acylation of 5-methoxyresorcinol (2.14) yielded a mixture of mono (2.15 and 2.17) and di (2.18) acylated products in 14:3 ratio (Figure 2.24). The mono acylation product comprised two regioisomers which were 2-methoxy (2.15) and 4-methoxyacetophenones (2.17) in 2:1 ratio.

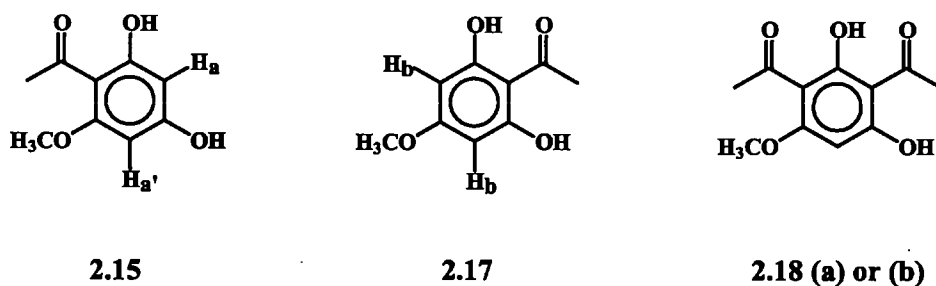


Figure 2.24 : Acylated products of monomethoxy phloroglucinol

The required product 2-methoxy-4,6-dihydroxyacetophenone (2.15) was readily isolated by dry column chromatography as a white crystalline solid in 42.8% yield and characterised by the analysis of its ¹H NMR spectrum in which aromatic protons H_a and H_{a'} resonated at δ 5.95 and 6.02 giving rise to

doublets, whereas protons H_b of 4-methoxyacetophenone (**2.17**) were observed as a singlet at δ 7.10.

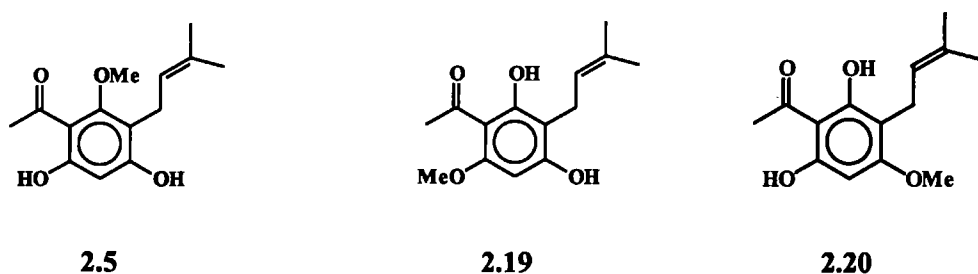


Figure 2.25 : Products of prenylation of **2.15 and **2.17****

BF₃-Et₂O promoted prenylation of 2-methoxy-4,6-dihydroxyacetophenone (**2.15**) with 2-methyl-but-3-ene-2-ol in dioxan yielded acronylin (**2.5**) and its regioisomer preremirol (**2.19**) in 12% and 10% yields respectively, whereas **2.17** yielded **2.20**. The regiochemistry of **2.5** was confirmed by means of NOESY correlations as depicted in Figure 2.26.

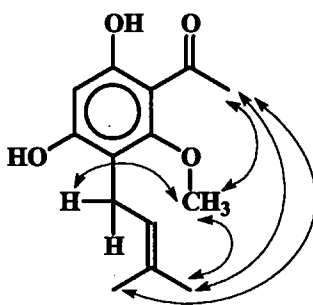


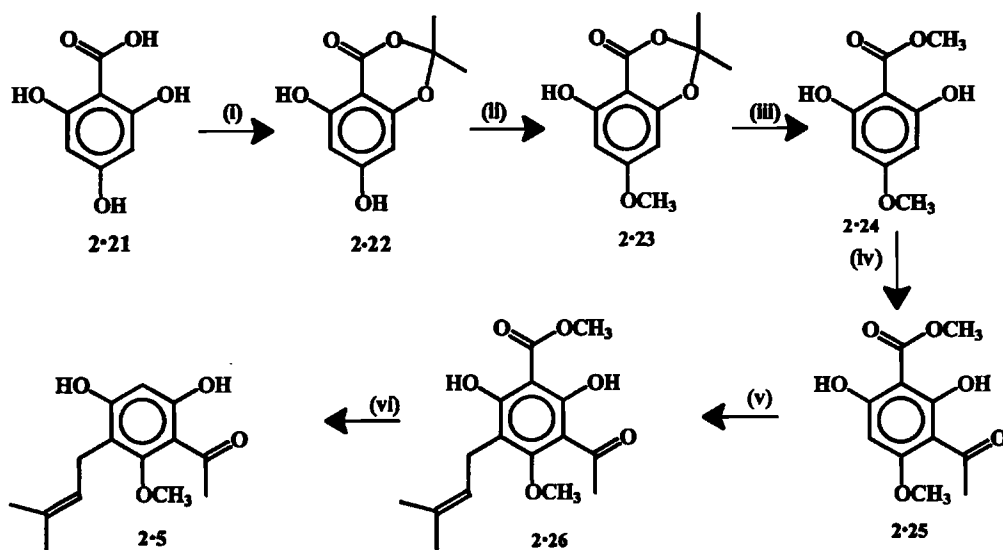
Figure 2.26 : NOESY correlations of acronylin

The synthesis of acronylin *via* this synthetic route was less than ideal due to the lack of regioselectivity and poor yields. An alternative method with high regioselectivity therefore, sought.

2.7.6 Regioselective synthesis of acronylin

(a) Approach 1

Regioselective synthesis of acronylin (**2.5**) was achieved, starting with commercially available 2,4,6-trihydroxybenzoic acid (**2.21**). The reaction of **2.21** with acetone in the presence of trifluoroacetic acid (TFA) and trifluoroacetic anhydride (TFAA) in THF¹⁵² gave the benzodioxin (**2.22**) in 95% yield. Since the hydroxyl group of **2.22** adjacent to the carbonyl group is hydrogen bonded, the mild methylation using Mitsunobu condition^{152, 153, 154,}
¹⁵⁵ gave exclusively the monomethylated ester **2.23** (Scheme 2.6).



Reagents: (i) TFA, TFAA, THF, acetone, 0-25 °C, 4 h; (ii) DEAD, PPh₃, MeOH, THF, 0 °C, 2 h; (iii) NaOMe, THF, Δ, 4 h; (iv) TiCl₄, AcCl, benzene, 0 °C-rt, 20 min; (v) prenyl bromide, 10% KOH, 2 h; (vi) 50% KOH, DMSO, Δ, 2 h.

Scheme 2.6 : Regioselective synthesis of acronylin

Acylation of **2.23** was unsuccessful with dioxin ring and therefore, compound **2.23** was treated with sodium methoxide (NaOMe) to remove the benzodioxin protection (Scheme 2.6). Although the previous researches used lithium methoxide (LiOMe) for the deprotection¹⁵², poor results were obtained, which could have been avoided by using NaOMe.

Acylation^{156, 157} of methyl ester (**2.24**) with titanium tetrachloride (TiCl₄) in benzene at 0 °C gave the corresponding acetophenone **2.25** in 91% yield (Scheme 2.6). The use of TiCl₄ as an acylation catalyst had several advantages including easy-work up procedure and higher yields compared to aluminium chloride (AlCl₃). The acyl group has equal possibilities to substitute at carbon positions 3 and 5. However, diacylated product was not obtained. The monoacylated product was isolated by dry column chromatography.

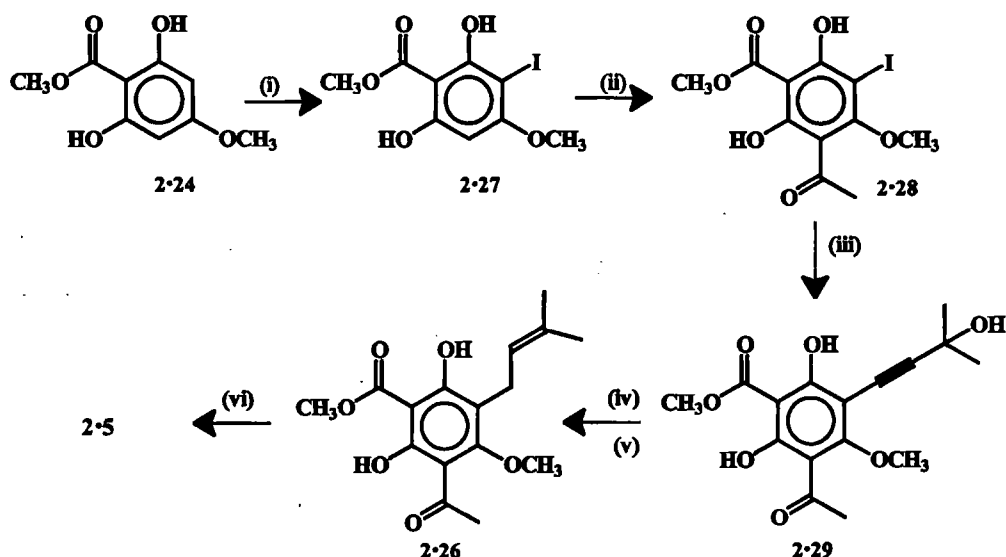
The isopentenyl group was introduced to the benzene ring of **2.25** by using previously described (In page 94) base catalysed reaction conditions (10% KOH and prenyl bromide) to afford the prenylated ether **2.26** in 30% yield (Scheme 2.6).

The final step, saponification and decarboxylation¹⁵⁸, was achieved by heating the prenylated ester **2.26** with aqueous KOH and dimethyl sulphoxide (DMSO) leading to acronylin (**2.5**) in 87% overall yield (Scheme 2.6). Even though this synthesis procedure gave acronylin in high regioselectivity, the

combined yields was less than 30%. Alternative methods for the synthesis of acronylin was therefore, sought.

(b) Approach 2

The reaction of aryl halides with terminal alkynes in the presence of Pd(0) has been reported for the formation of C-C bonds¹⁵⁹ and it appears to be applicable for the synthesis of prenylated phloroglucinols. Consequently, a palladium-catalysed coupling reaction was employed to obtain acronylin (2.5), (Scheme 2.7).



Reagents: (i) NIS, CH₃CN, 2 h; (ii) AcCl, TiCl₄, benzene, 2 h (iii) 2-methyl-but-3-yne-2-ol, PdCl₂(PPh₃)₂, CuI, Et₃N-DMF, 14 h; (iv) H₂, Pd/C, 3 h; (v) p TSA, toluene, reflux, 2 h; (vi) DMSO-KOH 130 °C, 1 h.

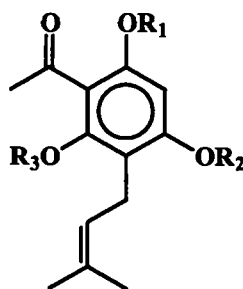
Scheme 2.7 : Pd(0) catalysed synthesis of acronylin

The use of iodo compounds is common in C-C bond formation reactions and their use in synthesis is mostly limited to either mono or poly methoxy phenols¹⁶⁰. The iodination of aryl ketones has been achieved using $\text{Ag}^+\text{CF}_3\text{COO}^-$ in previous work¹⁵⁹ for the synthesis of acronylin related compounds. The iodination of methyl-2,5-dihydroxy-4-methoxybenzoate (2.24) with N-iodosuccinimide (NIS) in acetonitrile¹⁶¹ at room temperature gave exclusively the mono iodomethyl benzoate, (2.27) which was then acylated to give (2.28). The coupling of the iodobenzoate (2.28)¹⁵⁹ with 2-methyl-but-3-yne-2-ol in the presence of dichloro-*bis*-triphenylphosphine palladium, CuI and PPh_3 in Et_3N -DMF under N_2 at 80 °C for 14 h afforded the desired methyl-2,5-dihydroxy-3-methoxy-4-(3-hydroxy-3-methylbutynyl)-benzoate (2.29) in 85% yield. The catalytic hydrogenation of 2.29 using Pd/C in methanol resulted a white solid which was treated with p-toluenesulphonic acid (pTSA) in toluene afforded 2.26. The compound 2.26 was decarboxylated¹⁵⁸ to yield acronylin (2.5) in 86% yield. Thus the Pd(0) coupling reaction for the synthesis of acronylin derivatives is a favoured method due to the high yield.

2.7.7 Synthesis of acronylin analogues

All possible analogues of acronylin (Figure 2.27) were needed as intermediates for SAR studies of acrovostone. The synthesis of acronylin (2.5) and analogues 2.6, 2.19 and 2.20 has already described. The regioselective

synthesis of the remaining analogues 2.30-2.32 are described in the following sections.



(2.5) $R_1, R_2 = H, R_3 = Me$: acronylin

(2.6) $R_1, R_2, R_3 = H$: Demethylacronylin

(2.19) $R_1 = CH_3, R_2 = R_3 = H$

(2.20) $R_2 = CH_3, R_1 = R_3 = H$

(2.30) $R_1, R_2 = CH_3, R_3 = H$

(2.31) $R_1, R_3 = CH_3, R_2 = H$

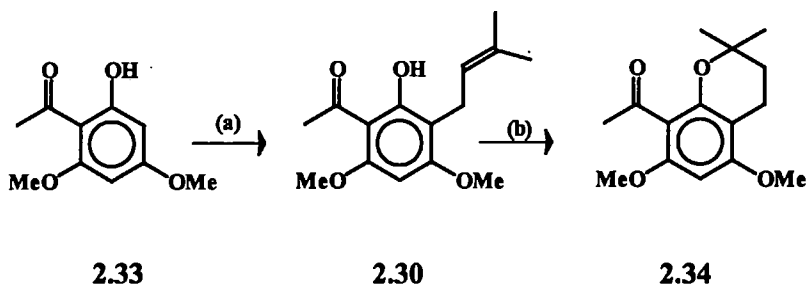
(2.32) $R_2, R_3 = CH_3, R_1 = H$

Figure 2.27 : Acronylin analogues

(a) Synthesis of 1-[4,6-dimethoxy-2-hydroxy-3-(3-methyl-but-2-enyl)-phenyl]-ethanone (2.30)

The methylation of 2,4,6-trihydroxyacetophenone using a Williamson arylmethyl ether synthesis¹⁶² afforded, 4,6-dimethoxy-2-hydroxyacetophenone (2.33) in 97% yield. 4,6-Dimethoxy-2-hydroxyacetophenone (2.33) was prenylated using the method previously described in literature¹⁵⁰. Although two products (2.30 and 2.32) are possible in this reaction, only one was

obtained. This was identified as **2.30** by cyclisation with Amberlyst 15 to give the corresponding chroman **2.34**. The regioselectivity observed may be due to the directing effects of the substituents of **2.33**.

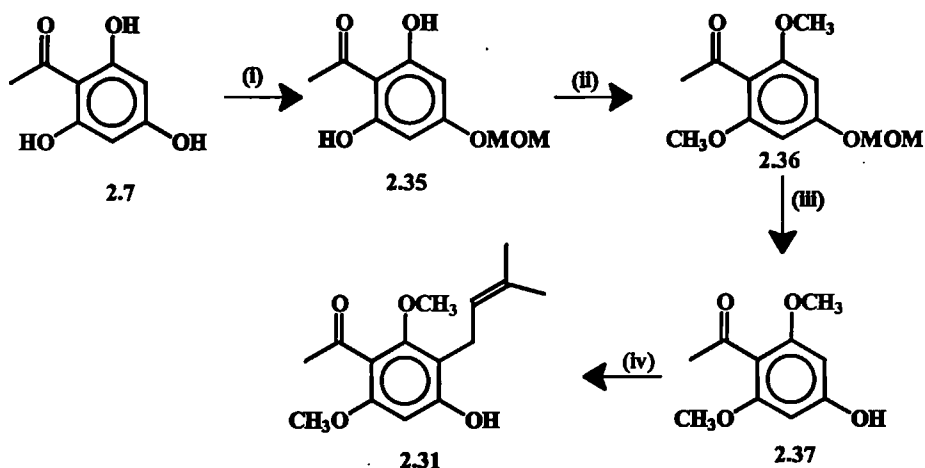


Reagents: (a) 2-methyl-but-3-ene-2-ol, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, dioxan, 40°C , 40 min; (b) Amberlyst 15, ether, reflux 2 h.

Scheme 2.8 : Synthesis of 2.30

(b) Synthesis of 1-[2-6-dimethoxy-4-hydroxy-3-(3-methyl-2-butenyl)-phenyl]-ethanone (2.31)

The synthesis of compound **2.31** was carried out in a regioselective manner as indicated in Scheme 2.9.



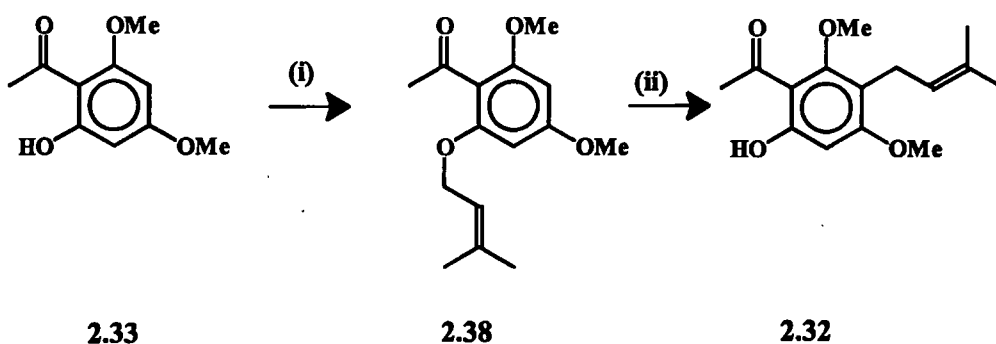
Reagents: (i) MOMCl, K_2CO_3 , acetone, reflux, 2 h; (ii) K_2CO_3 , Me_2SO_4 , acetone, reflux 3 h; (iii) 10% aq HCl, MeOH, reflux, 30 min; (iv) 2-methyl-but-3-ene-2-ol, $BF_3 \cdot Et_2O$, dioxane, 40 °C, 2 h.

Scheme 2.9 : Synthesis of acronylin analogue 2.31

The treatment of 2,4,6-trihydroxyacetophenone (**2.7**) with one equivalent of chloromethyl methyl ether (MOMCl) gave mixture of compounds. The desired compound **2.35** was isolated as a white crystalline solid in 45% yield. The dimethylation of **2.35** afforded **2.36** which was subjected to demethoxymethylation by treating with 10% aq HCl and methanol (1:5, v/v) to afford the dimethoxy compound **2.37** in 92% yield. The prenylation of **2.37** afforded **2.31** in 12% overall yield.

(c) **Claisen rearrangement for the synthesis of 1-[2,4-dimethoxy-6-hydroxy-(3-methyl-2-butenyl)-phenyl]-ethanone (2.32)**

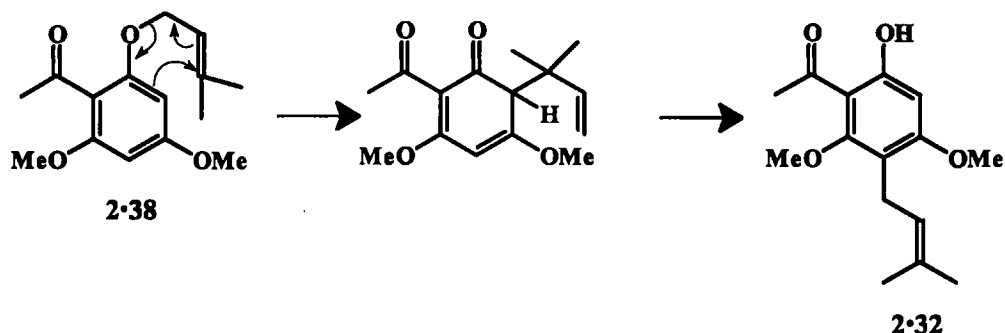
The applicability of the Claisen rearrangement for the synthesis of demethylacronylin (2.6) has been recently reported¹⁶³. Compound 2.32 which is the isomer of 2.30 has been synthesised *via* a Claisen rearrangement.



Reagents: (I) prenyl bromide, K₂CO₃, acetone, reflux, 15 h; (ii) 150 °C, 2 h.

Scheme 2.10 : Synthesis of acronylin analogue 2.32

The O-alkylation of dimethoxyacetophenone (2.33) in the presence of prenyl bromide and potassium carbonate exclusively gave O-prenylated product (2.38). The compound 2.38 was heated without solvent at 175 °C for 2 h to obtain 2.32 in 80% yield as showed in Scheme 2.10.



Scheme 2.11 : Claisen rearrangement of 2.38

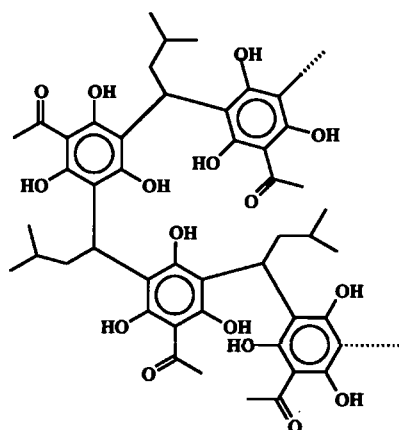
No intermediate product was isolated during Claisen rearrangement. It can be a concerted reaction which follows a 3,3-sigmatropic rearrangement¹⁶⁴ as depicted in Scheme 2.11.

2.7.8 Model reactions for the condensation of polyhydroxy phenols

Having synthesised the key intermediates demethylacronylin (2.6) and acronylin (2.5), the final step required their coupling with an isovaleryl moiety. In the present study, a series of model reactions was carried out to identify the most suitable conditions and reagents. The condensation of phenols with an aldehyde in the presence of acid to obtain *bis*-phenyl compounds has been widely studied¹⁶⁵. Among them *ortho*-linked methylene-*bis*phenols and hindered compounds have been received special attention due to their industrial application. These types of reactions have been previously reported under hydroxy alkylations and the synthesis of DDT is an example. An uncatalysed phenol-formaldehyde coupling reaction has also been reported which proceeds *via* salicyl alcohols¹⁶⁶. However, the utility of an uncatalysed

coupling for the acrovestone synthesis was limited due to the high reaction temperature required.

(a) Acid catalysed reaction



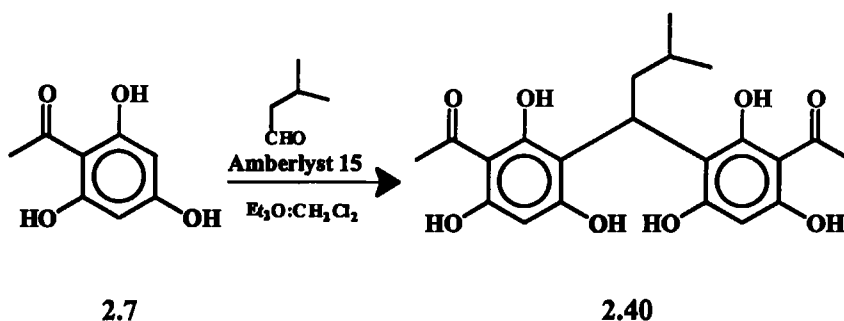
2.39

Figure 2.28 : Tentative structure for acetophenone polymer

As an approach to define a widely applicable route for the synthesis of acrovestone and its derivatives, condensation of 2,4,6-trihydroxyacetophenone with isovaleraldehyde has been examined under acidic conditions. The condensation was carried out in acidic media with various solvents diethyl ether, dichloromethane and THF. The reaction in diethyl ether was not successful with mineral acid at room temperature, but under reflux gave a polymeric product.

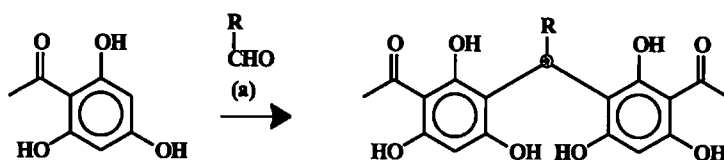
Similarly, condensation of 2,4,6-trihydroxyacetophenone (2.7) with isovaleraldehyde in the presence of Amberlyst 15 in diethyl ether was

attempted and at room temperature no product was observed, but product polymerised under reflux.



Scheme 2.12 : Condensation of acetophenone with isovaleraldehyde

The polymeric material was tentatively identified by means of its ^1H NMR spectrum in which the corresponding signals of **2.40** were poorly resolved. However, dichloromethane and Amberlyst 15 have been used for such phenolic condensations in literature¹⁴³, in the present study a mixture of dichloromethane:diethyl ether (4:1, v/v) was used. As a result of this slight modification of the solvent system, the required isovaleryl 2,4,6-trihydroxyacetophenone dimer **2.40** was obtained in 65% yield under reflux for 30 min (Scheme 2.12). Longer reaction time under reflux led to polymerisation, however, performing reaction at room temperature for 12 h gave the dimer **2.40** in 63% yield.



Scheme 2.13 : Condensation of acetophenones with aldehydes

Table 2.18 : Condensation of 2,4,6-trihydroxyacetophenone with aldehydes

| Compound No | R | Yield (%) ^a |
|-------------|---|------------------------|
| 2.41 | H | 80 |
| 2.42 | CH ₃ | 85 |
| 2.43 | CH(CH ₃) ₂ | 78 |
| 2.44 | CH ₂ CH ₂ CH ₃ | 73 |

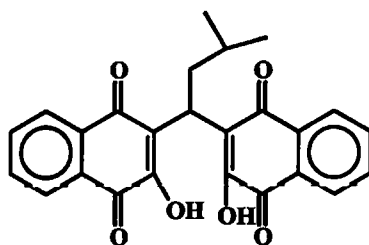
^a Yields were based on isolated products.

By utilising these conditions defined for the coupling of two electron rich phenols with an aldehyde, a series of novel compounds (2.41-2.44) were synthesised by varying the aldehyde (Scheme 2.13 and Table 2.18).

(b) Base catalysed reaction

Reports of the condensation of electron rich aromatic systems with aldehydes in the presence of base are limited in number. Recent reports for the synthesis of the naphthoquinone dimer (2.45) with aldehydes gave unexpected results

¹⁶⁷. The same methodology was adapted for the synthesis of acetophenone dimers in the present study. The reaction was carried out over two days in DMF in the presence of Et₃N at 40 °C and lead to the acetophenone dimer **2.40** (In page 65). The long reaction time and the tedious workup procedure discouraged further studies of base catalysed reactions.

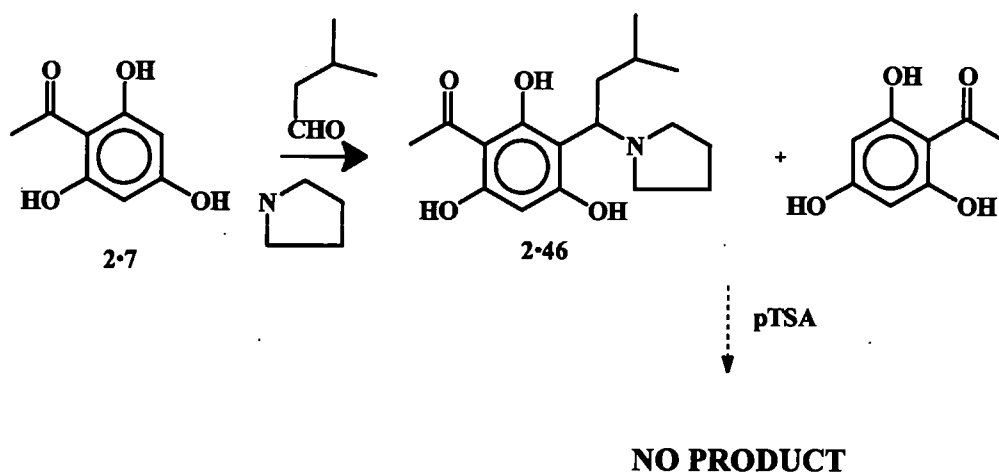


2.45

Figure 2.29 : Naphthoquinone dimer

(c) Pyrrolidine coupling approach

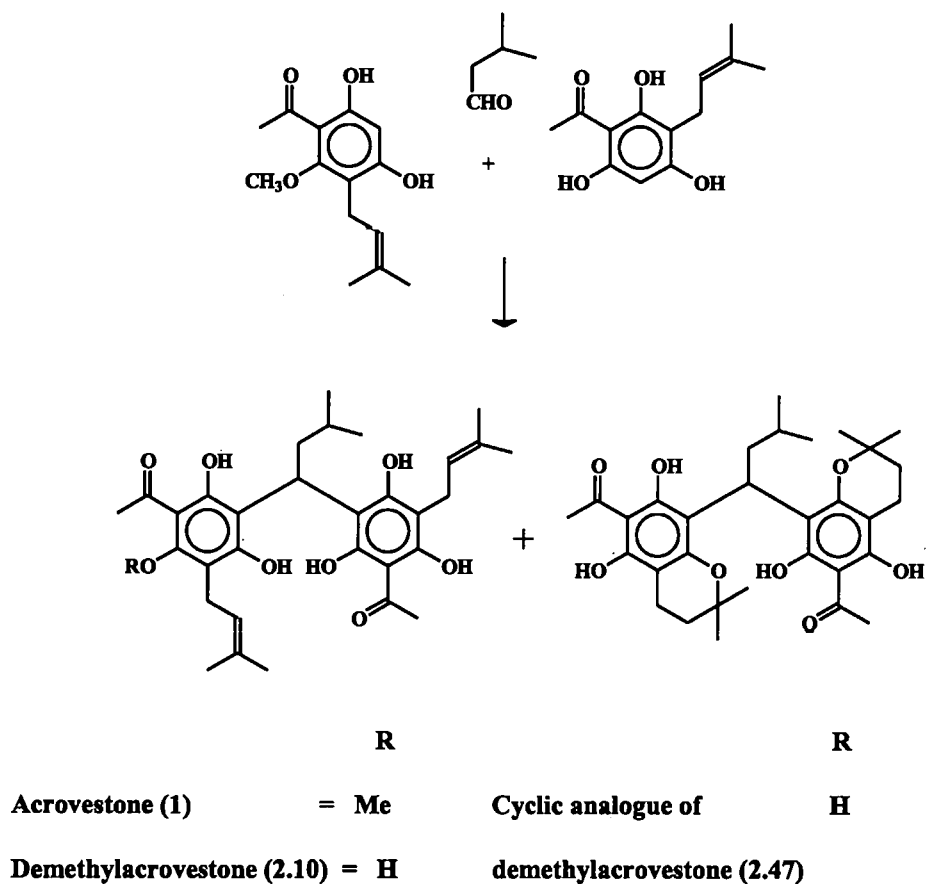
The use of pyrrolidine adducts has been reported for the synthesis of bio-active phloroglucinols¹⁶⁸. Attempts to prepare the corresponding acetophenone-pyrrolidine adducts (**2.46**) failed at ice cold temperature (Scheme 2.14). However, a yellow solid was obtained at -78 °C. The reaction of this with acetophenone failed to give the corresponding dimer.



Scheme 2.14 : Attempt to synthesis of acetophenone dimer *via* pyrrolidine adduct

2.7.9 Synthesis of acrovestone and demethylacrovestone

In order to complete the synthesis of acrovestone, equivalent amounts of acronylin (2.5) and demethylacronylin (2.6) were reacted with isovaleraldehyde in the presence of Amberlyst 15 in a mixture of dichloromethane and diethyl ether (4:1, v/v). The reaction was completed within 20 min under reflux yielding three compounds (Scheme 2.15) which were identified as acrovestone (1), demethylacrovestone (2.10) and a novel cyclic analogue of demethylacrovestone (2.47). The separation of these compounds was achieved by dry column chromatography affording the compounds in 30%, 15% and 15% yields, respectively. The spectroscopic data of 1 and 2.10 was compatible with those of the natural products (Table 2.3, in page 9)^{104, 109}. This is the first report of the synthesis of acrovestone (1) and demethylacrovestone (2.10).



Scheme 2.15 : Synthesis of acrovestone and demethylacrovestone

After the synthesis of acrovestone and demethylacrovestone, the work was extended towards the synthesis of analogues of acrovestone by changing the substituent pattern of aryl ketone units and aldehyde as generalised in Scheme 2.16.



Scheme 2.16 : General reaction for synthesis of acrovestone analogues

Symmetrical dimers could be readily synthesised by condensing two molecules of monomers with a corresponding aldehyde under acidic conditions. However, the chroman **2.47** formation is highly favourable when demethylacronylin is used as one of the monomers. The methoxy group(s) of the acronylin analogue prevents the formation of symmetrical dimers and instead facilitate the formation of unsymmetrical compounds. The yields of the final products vary with the type of monomer. In general, the synthesis of acrovestone related unsymmetrical dimers are achievable.

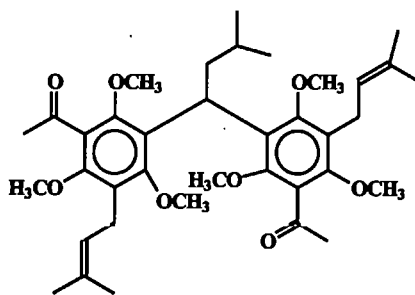
The main difficulty associated with this is the formation of a mixture of compounds. But this mixture is separable by chromatography. In the present study the product optimisation and chiral synthesis have not been addressed.

2.7.10 Semisynthetic derivatives of acrovestone

In the presesnt study several semisynthetic derivatives of acrovestone have been made to investigate the importance of hydroxyl groups for the insecticidal activity.

(a) Methyl ether derivatives

Per methylation of acrovestone was achieved using dimethyl sulphate and potassium carbonate¹⁶² to yield pentamethoxy acrovestone (**2.48**) (Figure 2.30). The peaks were more resolved in the ¹H NMR spectrum of **2.48** compared to that of acrovestone.

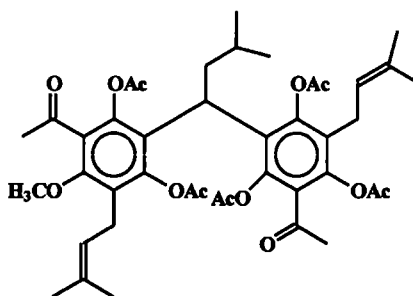


2.48

Figure 2.30 : Pentamethoxy acrovestone

(b) Acetyl derivatives

Acrovestone penta-acetate (2.49) (Figure 2.31) has been synthesised using a standard acetylation procedure¹⁰⁶.



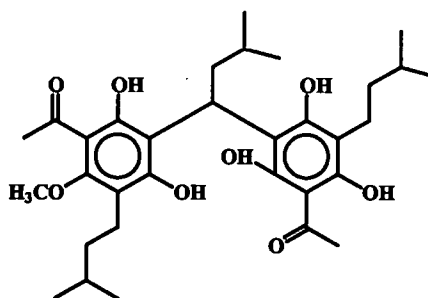
2.49

Figure 2.31 : Acrovestone penta-acetate

(c) Hydrogenation

Acrovestone was hydrogenated using Pd/C in ethyl acetate¹⁰⁶. The tetrahydroacrovestone (2.50) was obtained as a yellow crystalline solid. The

spectral data of **2.50** were in good agreement with those previously reported for tetrahydroacrovestone¹⁰⁶.

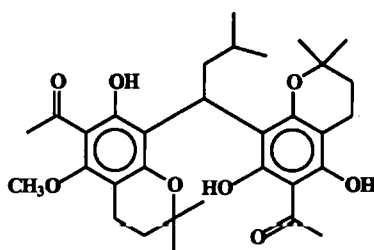


2.50

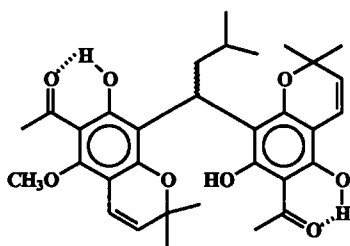
Figure 2.32 : Tetrahydroacrovestone

(d) Cyclic derivatives

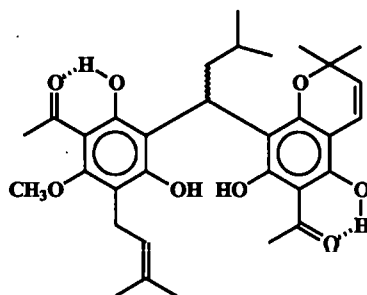
The cyclic analogue of acrovestone, chroman (**2.51**), was obtained with Amberlyst 15 under reflux for 6 h. The reaction time for the formation of chroman of acrovestone was longer than anticipated compared to that of demethylacrovestone. During the condensation of demethylacronylin and acronylin, chroman of demethylacrovestone (**2.47**) was obtained (In page 111) but not **2.51** (Figure 2.33).



2.51



2.52



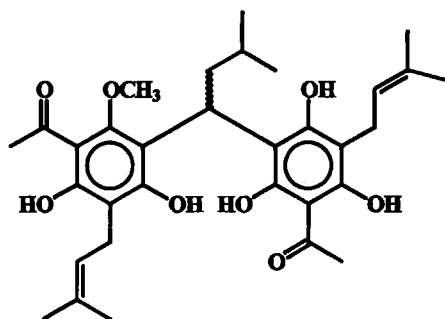
2.53

Figure 2.33 : Cyclic analogues of acrovestone

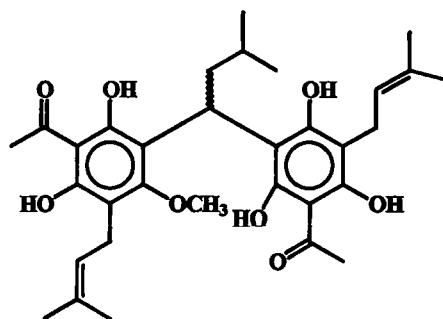
The reaction of acrovestone with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)¹⁵⁸ gave two chromenes, **2.52** and **2.53**. They were identified by ¹H NMR spectroscopy as mono (**2.53**) and di (**2.52**) chromenes (Figure 2.33).

2.7.11 Synthesis of methoxy analogues of acrovestone

The position of the methoxy group of acrovestone has been confirmed by X-ray analysis by T.S wu *etal*¹⁰⁴. In the present study, we reconfirmed this by performing long range hetero COSY experiment and total synthesis.



2.54

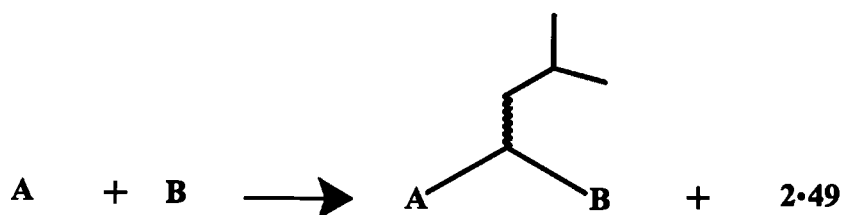


2.55

Figure 2.34 : Regioisomers of acrovestone

A series of acrovestone analogues **2.54-2.59** (Table 2.19) was synthesised, varying the position as well as the number of methoxy group, i.e. appropriate acronylin analogues (**2.5**, **2.6**, **2.19**, **2.20**, **2.30**, **2.31**, **2.32**, in page 101) were condensed with isovaleraldehyde. The yields of asymmetric acrovestone analogues were optimised by refluxing the reaction mixture for a longer period. In this synthesis demethylacrovestone (**2.10**) was easily cyclised to give chroman **2.47**.

Table 2.19 : Synthetic analogues of acrovestone



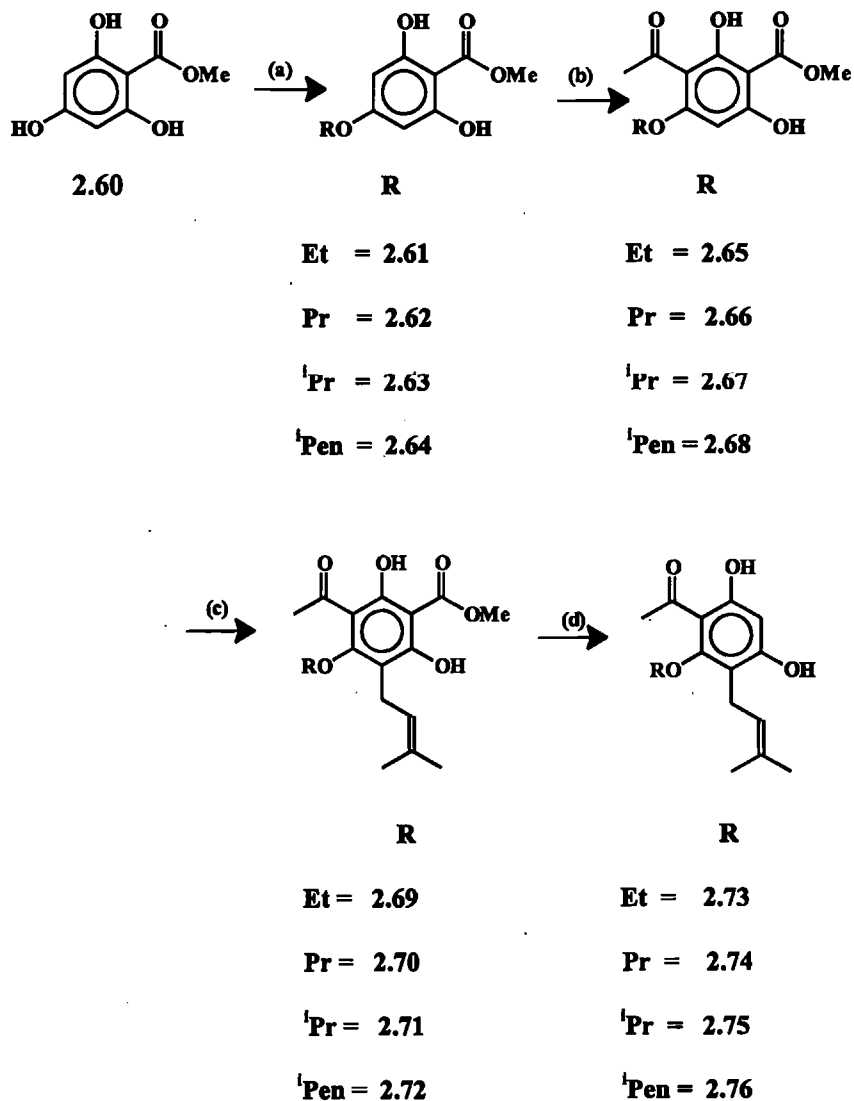
| A | B | Acrovestone analogue (% yield) ^a | Chroman (% yield) ^a |
|------|-----|---|--------------------------------|
| 2.19 | 2.6 | 2.54 (30) | (40) |
| 2.20 | 2.6 | 2.55 (30) | (30) |
| 2.5 | 2.5 | 2.56 (22) | - |
| 2.32 | 2.6 | 2.57 (25) | (45) |
| 2.33 | 2.6 | 2.58 (25) | (35) |
| 2.31 | 2.6 | 2.59 (25) | (40) |

^a Yields were based on isolated products.

Compounds 2.54-2.59 are novel analogues of acrovestone all of which are light yellow crystalline solids stable at room temperature.

2.7.12 Synthesis of acrovestone analogues

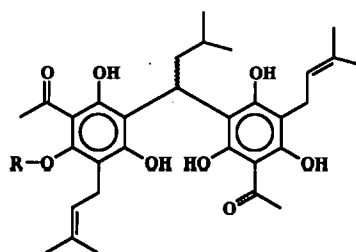
(a) Variations of alkoxy side chain



Reagents:. (a) DEAD, PPh₃, ROH, THF 0 °C; (b) TiCl₄, AcCl, benzene; (c) 2-methyl-but-3-ene-2-ol, BF₃-E₂O, dioxane, 40 °C, 2 h; (d) 50% KOH-DMSO, reflux.

Scheme 2.17 : Alkoxy variations of acronylin

A series of acronylin analogues (**2.73-2.76**) was synthesised (Scheme 2.17) using methodology similar to that used for the synthesis of acronylin (in page 56, Approach 1). The methyl benzoate **2.60** was subjected to Mitsunobu conditions with different alcohols to obtain a series of ethers (**2.61-2.64**). TiCl_4 promoted acylation of these ethers gave the corresponding acylated compounds (**2.65-2.68**). Prenylation of acylated ethers resulted in compounds, **2.69-2.72** in 10-15% yield. Finally, decarboxylation yielded the required analogues, **2.73-2.76** in 70-75% yields (Scheme 2.17). Each analogue synthesised by varying the alkoxy side chain was condensed with demethylacronylin (**2.6**) to give the corresponding acrovestone analogues **2.77-2.80** (Figure 2.35).



| Compound | R |
|-------------|------------------|
| 2.77 | Et |
| 2.78 | Pro |
| 2.79 | ^t Pro |
| 2.80 | ^t Pen |

Figure 2.35 : Alkoxy variations of acrovestone

The cyclic analogue of demethylacrovestone (**2.47**) was obtained as a major byproduct in this synthesis and was easily separated by dry column flash

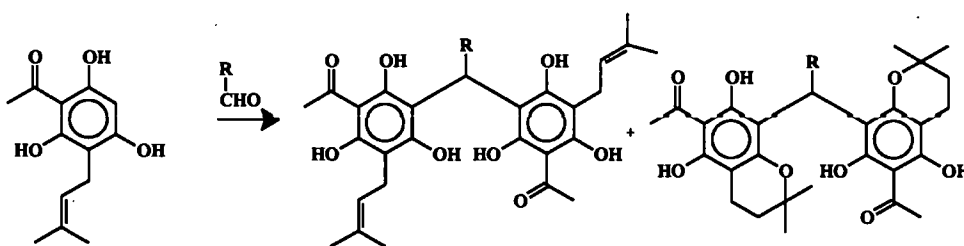
chromatography. The yields of synthetic analogues **2.77-2.80** were in range of 20%-30%. Compounds **2.77-2.80** were found to be novel structures.

2.7.13 Synthesis of demethylacrovestone analogues

(a) Variations of central alkyl group

Demethylacronylin was condensed under reflux with a series of aldehydes (formaldehyde, acetaldehyde, propanaldehyde, isopropanaldehyde, butanaldehyde) in the presence of Amberlyst 15, to yield compounds **2.81-2.85** (Table 2.21).

Table 2.21 : Analogues of demethylacrovestone



| R | Product | (Yield %) | Chroman | (Yield %) |
|---|-------------|-----------|-------------|-----------|
| H | 2.81 | (23) | 2.86 | (50) |
| CH ₃ | 2.82 | (20) | 2.87 | (65) |
| C ₂ H ₅ | 2.83 | (25) | 2.88 | (45) |
| CH(CH ₃) ₂ | 2.84 | (22) | 2.89 | (68) |
| CH ₂ CH ₂ CH ₃ | 2.85 | (15) | 2.90 | (40) |

The formation of corresponding chromans was preferred with all these aldehydes. The compounds **2.81-2.85** are novel.

(b) Variations in acyl group

2,4,6-Trihydroxyisovalerophenone (**2.91**) and isobutyrylphenone (**2.92**) were synthesised starting from commercially available phloroglucinol following the standard AlCl_3 promoted Friedel-Crafts acylation reaction.

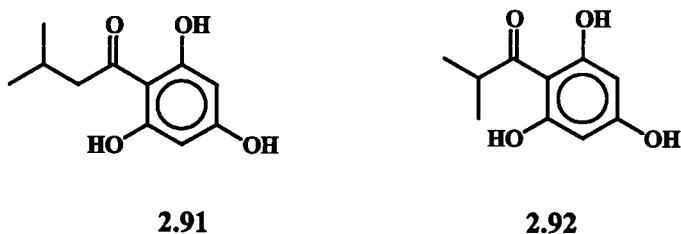


Figure 2.36 : Isovalero and isobutyrylphenones

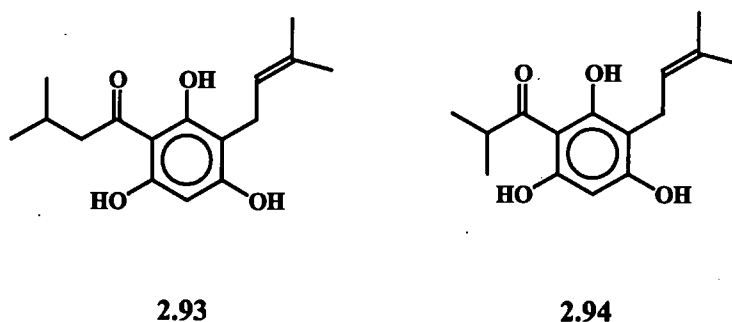


Figure 2.37 : Isopentenyl aryl ketones

They were converted to corresponding prenylated phenones **2.93** and **2.94**, the condensation of which with demethylacronylin gave compounds **2.95-2.98**

(Figure 2.38). The synthesised compounds were characterised by means of ^1H and ^{13}C NMR spectroscopy.

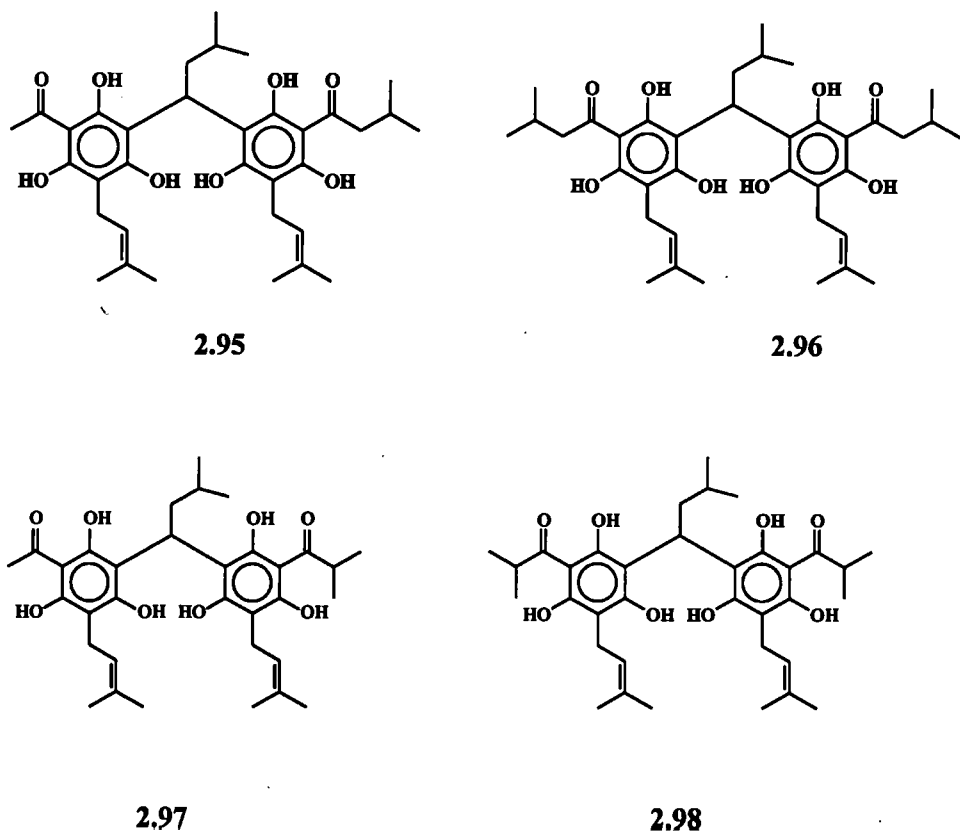


Figure 2.38 : Variations of aryl ketone side chain of demethylacrovestone

2.8 SYNTHESIS OF ACROVESTONE-KUNZEIN HYBRID MOLECULES

2.8.1 Introduction

Phloroglucinols are widely distributed in higher plants and known to possess a vast spectrum of biological activities¹³⁹. The antibiotic, antiviral and phytotoxic activities of these are well documented¹⁶⁹. The vast spectrum of

structural diversity of phloroglucinols provides new bio-active compounds for synthetic studies.

The antibacterial β -tricarbonyl compounds, uliginosin A (2.99), uliginosin B (2.100)¹⁷⁰ (Figure 2.39), robustadials¹⁷¹ and related macrocarpals¹⁷² have been recently isolated and some synthesis studies have been carried out.

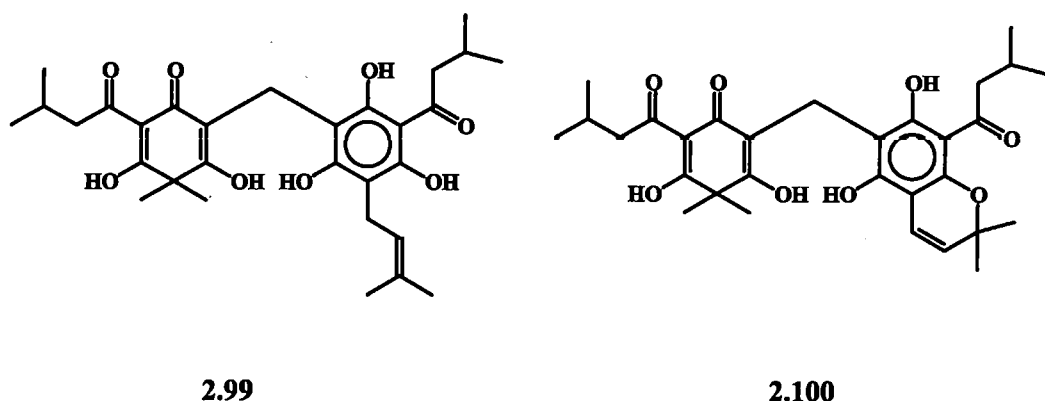
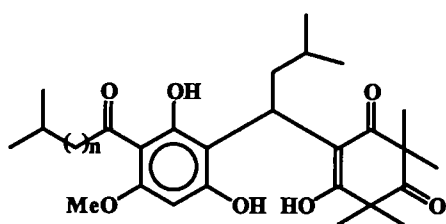


Figure 2.39 : Uliginosin A and uliginosin B

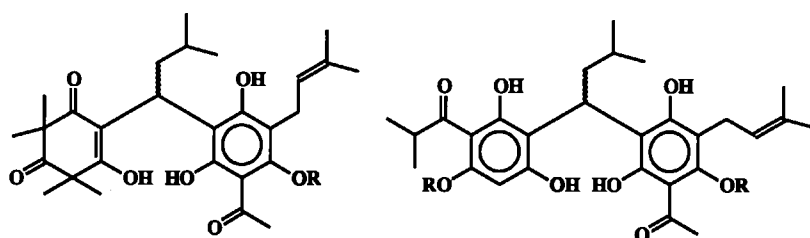
Recent chemotaxonomic studies of the genus *Kunzea* revealed the presence of insecticidal phloroglucinol derivatives in species of *Kunzea* (2.101 and 2.102) (Figure 2.40)¹⁷³. The insecticidal activity of kunzein compounds was widely studied¹⁷⁴, and reported to be comparable with that of acrovestone. These compounds (Figure 2.40) showed structural similarities to acrovestone.



| n | Compound |
|---|----------|
| 0 | 2.101 |
| 1 | 2.102 |

Figure 2.40 : Kunzeins from Family Myrtaceae

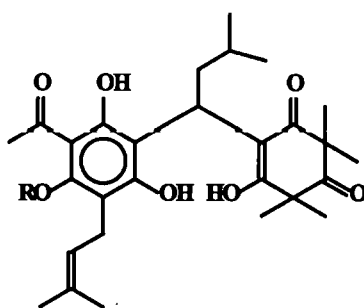
The present study required all four hybrid compounds (2.103-2.106) incorporating features of both acrovestone and kunzein (0) (Figure 2.41).



| R | Compound | R | Compound |
|-----------------|----------|-----------------|----------|
| H | 2.103 | H | 2.105 |
| CH ₃ | 2.104 | CH ₃ | 2.106 |

Figure 2.41 : Acrovestone-kunzean hybrid compounds

2.8.2 Synthesis of acro-kunzein hybrid compounds, 2.103 and 2.104



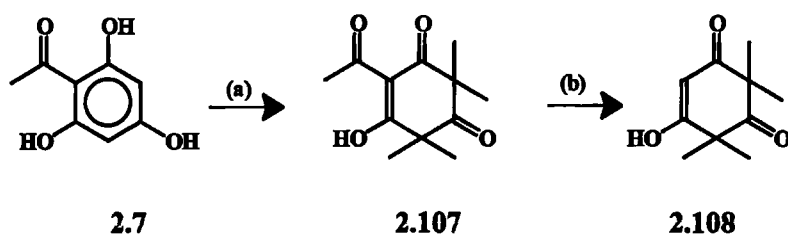
R

2.103 = H

2.104 = CH₃

Figure 2.42 : Prenylated kunzeins

The synthesis of compounds **2.101** and **2.102** has been recently reported and their insecticidal activity was measured¹⁷⁴. Synthesis of one of the sub structural units, syncarpic acid (**2.108**), was achieved using a previously reported procedure¹⁷⁴ in two steps starting from 2,4,6-trihydroxyacetophenone (**2.7**) (Scheme 2.18). Treatment of **2.7** with NaOMe and methyl iodide (MeI) gave the corresponding alkylated β -diketone¹⁶⁸ (**2.107**). Deacylation of this compound with concentrated sulphuric acid gave syncarpic acid (**2.108**) in good yield.



Reagents: (a) NaOMe, MeOH, MeI; (b) 50% H₂SO₄-H₂O.

Scheme 2.18 : Synthesis of syncarpic acid

The next step was the coupling of syncarpic acid with acronylin and demethylacronylin to obtain the corresponding acrovestone-kunzean hybrid compounds, **2.103** and **2.104**, respectively (Figure 2.42).

The reaction between syncarpic acid (**2.108**) and formaldehyde in the presence of 1% KOH has been reported to give the *bis*-compound **2.109** in good yield¹⁷⁴ (Figure 2.43).

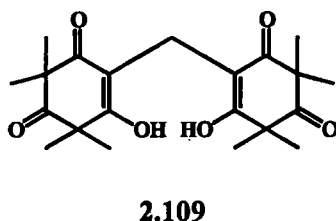
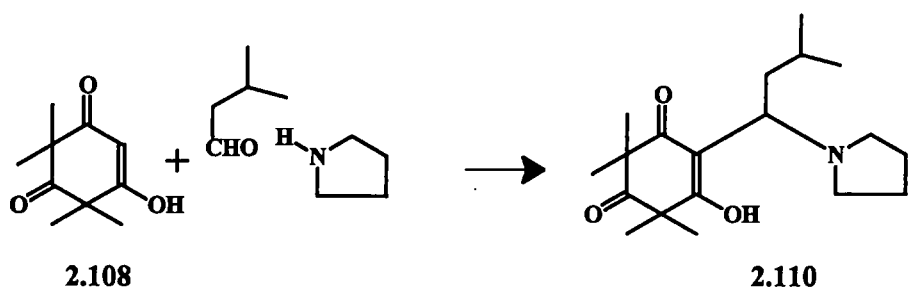


Figure 2.43 : *Bis*- compound

Reaction of pyrrolidine with syncarpic acid (**2.108**) in the presence of isovaleraldehyde gave the pyrrolidine adduct (**2.110**) by suppressing the formation of the corresponding *bis*-compound¹⁷⁵. This facilitated the coupling

between the prenylated aryl ketone and the β -diketone syncarpic acid (Scheme 2.19).



Scheme 2.19 : Synthesis of pyrrolidine adduct

The ^1H NMR spectrum of the Mannich base (2.110) indicated that all protons of the pyrrolidine ring were non-equivalent. This can be rationalised by considering the hydrogen bonding between the enol hydrogen and the nitrogen atom which holds the pyrrolidine ring in a preferred conformation, perpendicular to the syncarpic acid moiety (Figure 2.44).

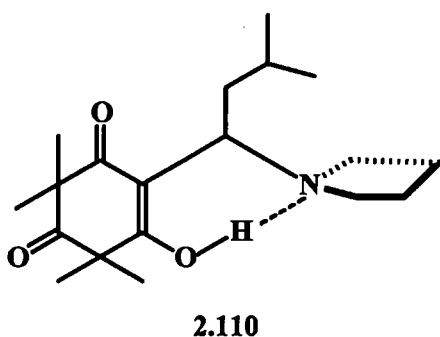
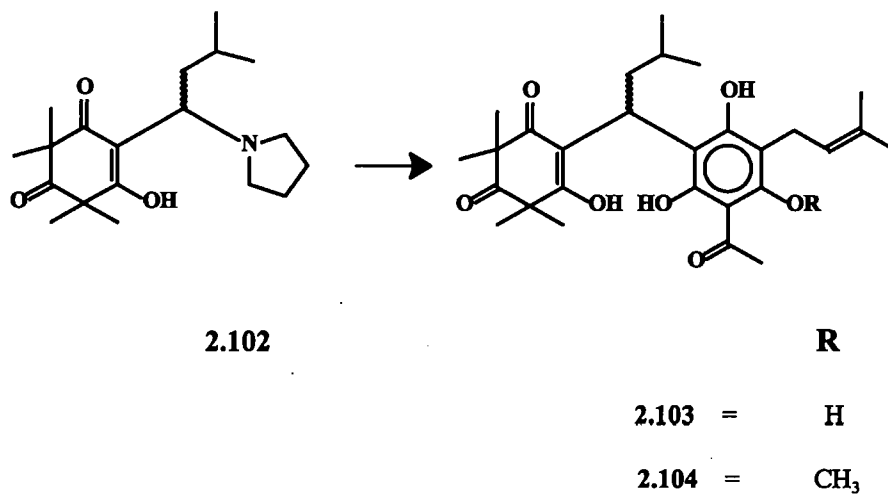


Figure 2.44 : Conformation of the Mannich base

The coupling of syncarpic acid and the aryl ketones, acronylin and demethylacronylin was achieved in 40-46% yield using pTSA as a catalyst at

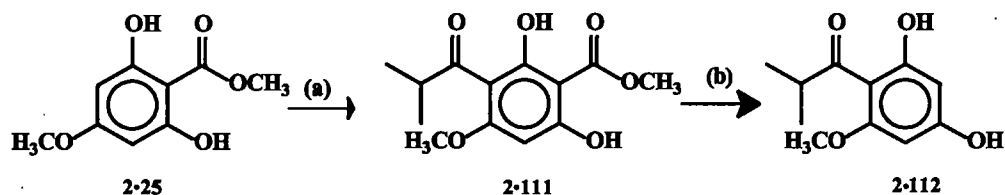
ice cold temperature in dry THF¹⁷⁵ (Scheme 2.20). The acrovestone-kunzein compounds (2.103 and 2.104) are novel.



Reagents: (a) acronylin or demethylacronylin, pTSA, THF, 0 °C → 20 °C

Scheme 2.20 : Coupling of the Mannich base with aryl ketones

2.8.3 Synthesis of acro-kunzein hybrid compounds, 2.105 and 2.106

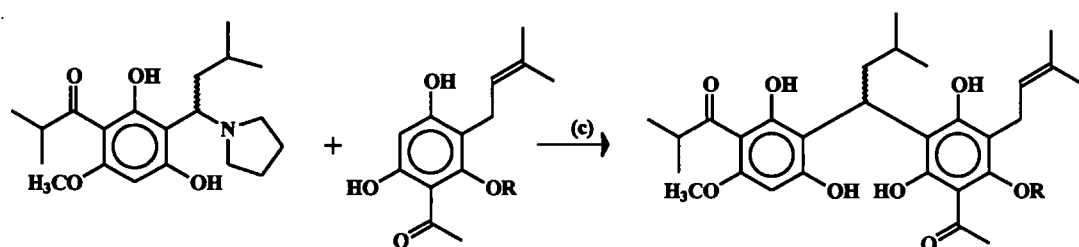


Reagents: (a) Isobutyryl chloride, TiCl₄, benzene; (b) KOH-DMSO, 130 °C.

Scheme 2.21 : Regioselective synthesis of the 2-methoxy-isobutyrolophenone

The synthesis of 2-methoxy-4,6-dihydroxyisobutyrophenone (2.112) was carried out in a regioselective manner illustrated in Scheme 2.21. This was coupled with acronylin (2.6) and demethylacronylin (2.6) to yield required

hybrid compounds **2.105** and **2.106**. Although the desired products are **2.105** and **2.106** by Amberlyst assisted condensation reaction there was a possibility of forming two regioisomers of **2.105** and **2.106**.



2.113

| | R | | R |
|------------|-----------------|--------------|-----------------|
| 2.5 | H | 2.105 | H |
| 2.6 | CH ₃ | 2.106 | CH ₃ |

Reagents: (a) pTSA, diethyl ether, rt.

Scheme 2.22 : Regioselective synthesis of acro-kunzean hybrid compounds.

The coupling of acronylin (**2.5**) and demethylacronylin (**2.6**) with 2-methoxy-isobutyrolophenone (**2.112**) was achieved in a regioselective manner by making the pyrrolidine adduct of isobutyrolophenone **2.113** as described in literature¹⁷⁶. The novel compounds **2.105** and **2.106** were obtained as light yellow crystalline solids in 20% , 25% yields, respectively.

2.9 SYNTHESIS OF CONSTRAINED-ACROVESTONE ANALOGUES

2.9.1 Introduction

In acrovestone the two phloroglucinol moieties have restricted rotation due to the intramolecular hydrogen bonding. However, the single crystal X-ray analysis has shown the molecule is not planer. To further, investigate the importance of the overall shape for activity, constrained compounds were sought.

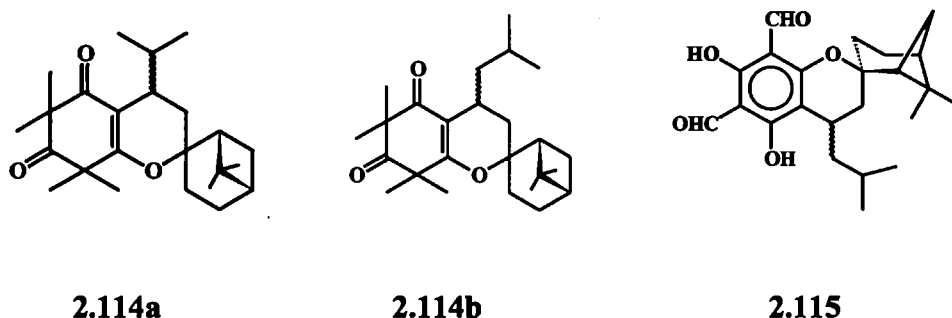
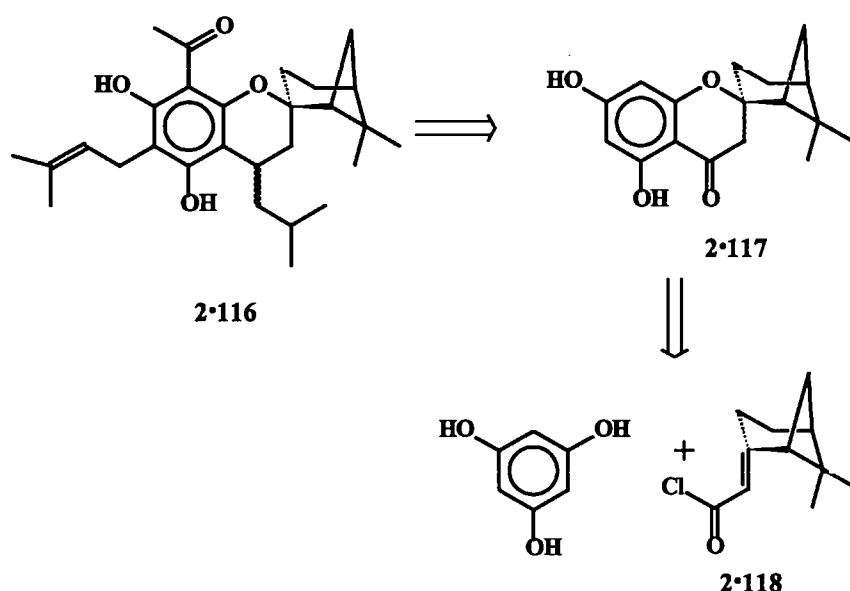


Figure 2.45 : Natural products with

The choice of target compound 2.116 was based on previously reported insecticidal natural products 2.114a, 2.114b¹⁷⁴ and robustadial (2.115)¹⁷⁷⁻¹⁸⁰ (Figure 2.45). The compound 2.115 has not been investigated for insecticidal activity but known for anti-plasmodium activity¹⁴⁰.

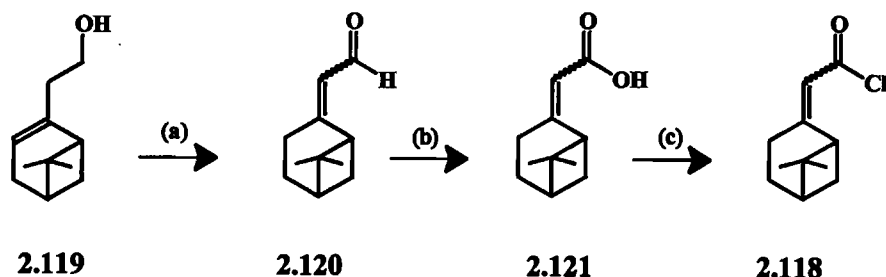
2.9.2 Synthesis of prenylated derivatives of robustadials

Synthesis of target compound **2.116** was based on a recently reported synthetic approach (Scheme 2.23)¹⁸¹ for robustadials. According to the retrosynthetic analysis the chromanone **2.117**, a key intermediate had to be constructed. The chromanone **2.117** was synthesised in four steps starting with enantiomerically pure (-)-nopol (**2.119**).



Scheme 2.23 : Retrosynthetic analysis of robustadial analogues

Direct conversion of (-)-nopol (**2.119**) to the corresponding acid using Jones reagent gave complicated mixtures. (-)-Nopol (**2.119**) was therefore converted to the corresponding aldehyde **2.120** which was obtained as a mixture of 4:1 (*E*) and (*Z*) isomers under Swern oxidation reaction conditions¹⁸¹ (Scheme 2.24).



Reagents: (a) Oxalyl chloride, DMSO, Et₃N, CH₂Cl₂; (b) AgNO₃, NaOH, EtOH; (c) Oxalyl chloride, benzene.

Scheme 2.24 : Synthesis of the acid chloride of nopol

The simultaneous double bond shift to give the α,β unsaturated systems was observed during the Swern oxidation. The presence of an isomeric mixture was evident by two sets of doublets appearing at δ 9.10 and 9.20 in the ¹H NMR spectrum of the aldehyde (2.120).

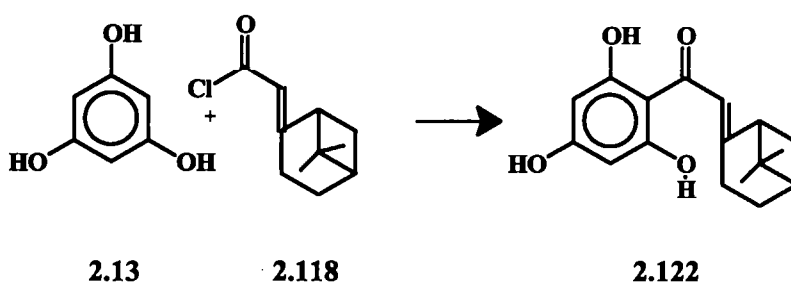


Figure 2.46 : *E* and *Z* isomers of 2.120

The configuration of this isomeric mixture of aldehydes was determined essentially on the evidence from the ¹H NMR spectrum. In the ¹H NMR spectrum, the allylic bridgehead proton appeared as a triplet at δ 2.45 and 3.45 corresponding to the major and minor isomers, respectively. The latter isomer

was assigned as *Z* due to the deshielding effect of the carbonyl group (Figure 2.46).

Treatment of **2.120** with ethanolic AgNO_3 and NaOH gave the *E* isomer of the acid of nopol (**2.121**). The corresponding acid chloride of 6,6-dimethylbicyclo [3.1.1] heptan-2-ylidene-acetic acid (**2.121**) was obtained by treating the acid with oxalyl chloride in benzene (Scheme 2.24).



Reagents: (d) ZnCl_2 , CH_2Cl_2 .

Scheme 2.25 : Synthesis of intermediate 2.122

Friedel-Craft acylation with acid chloride (**2.118**) and phloroglucinol (**2.13**) in the presence of zinc chloride (ZnCl_2) gave the aryl ketone **2.122** (Scheme 2.25). A singlet at δ 6.85 in the ^1H NMR spectrum of intermediate **2.122** showed that the double bond remained exocyclic during the reaction.

The intermediate, **2.122** was converted to the corresponding chromanone **2.117** in 95% yield by refluxing with ethanolic K_2CO_3 for 2 h. The ring closure of **2.122** was found to be stereoselective. The stereoselectivity of this oxy-Michael ring closure has been examined previously by Majewski *et al*¹⁸². The

trans product of chromanone **2.117** can be obtained using a weak base as a catalyst. Purification of the aryl ketone intermediate, **2.122** was not necessary for the formation of chromanone **2.117** which was obtained in good yield (Figure 2.47).

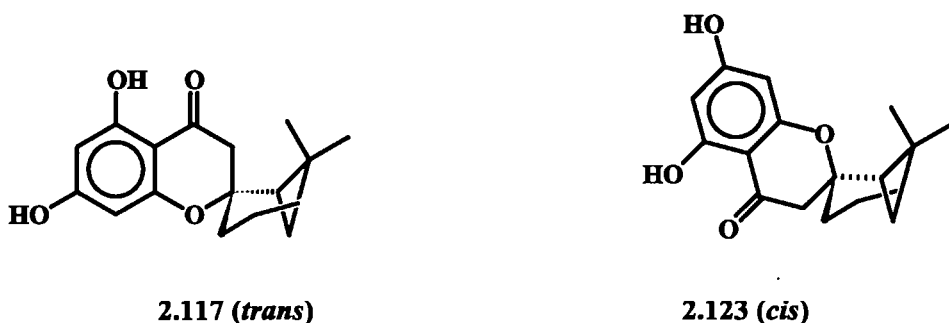
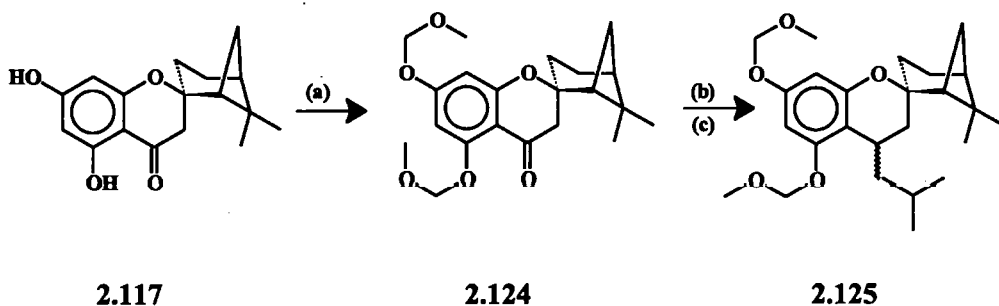


Figure 2.47 : *Cis* and *trans* isomers of chromanone

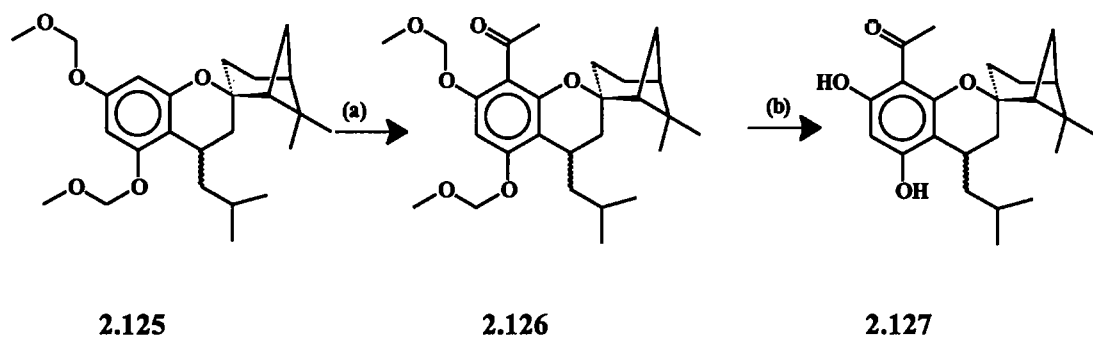
The next step is the introduction of the isobutyl group to the carbonyl group of **2.117**. The two hydroxyl groups were protected as methoxy methyl ethers to afford **2.124**. Grignard addition followed by hydrogenation of **2.124** gave an isomeric mixture of **2.125** in 98:2 ratio and the major isomer was obtained as a white solid (Scheme 2.26).



Reagents (a) MOMCl, K_2CO_3 , acetone, reflux, 2 h; (b) $i\text{BuMgCl}$, THF, -20°C , N_2 , 2h; (c) 10 % Pd/C, ethanol, H_2 , 3 h, rt.

Scheme 2.26 : Synthesis of compound 2.125

TiCl₄ promoted acylation introduced the acyl group to the aromatic ring of **2.125** to give **2.126** (Scheme 2.27).



Reagents: (a) Acetyl chloride, TiCl₄, benzene, 0 °C- rt; (b) 10 % aq HCl, MeOH, reflux, 30 min.

Scheme 2.27 : Synthesis of intermediate 2.127

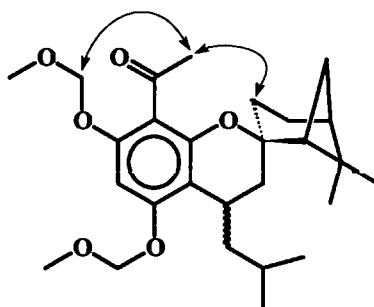
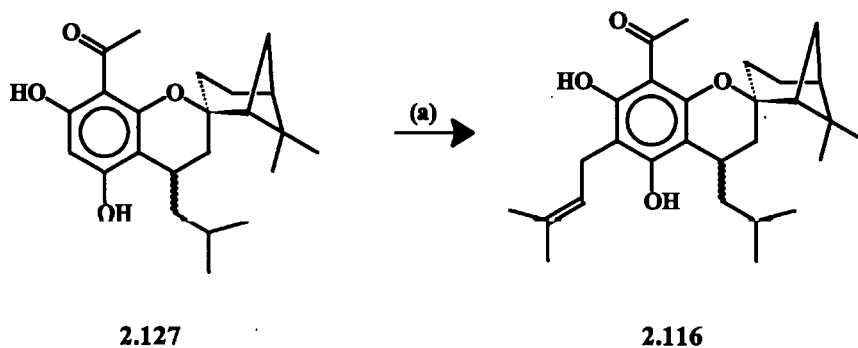


Figure 2.48 : Selected NOESY correlation of compound 2.126

The position of the acyl group of **2.126** was determined by performing an nOe experiment (Figure 2.48). The positive nOe interactions of methyl protons of acyl group (δ_H 2.61) and the protons resonating at δ_H 2.3 and 1.93 of the pinene moiety confirmed the location of the acetyl group as indicated in Figure 2.47.



Reagents: (a) 2-methyl-but-3-ene-2-ol, $\text{BF}_3\text{-Et}_2\text{O}$, dioxane, 40°C , 2 h.

Scheme 2.28: Synthesis of compound 2.116

The synthesis of **2.127** was achieved in 60% yield by demethoxymethylation of **2.126** with a mixture of 12% aqueous HCl and methanol (1:5, v/v) (Scheme 2.27). $\text{BF}_3\text{-Et}_2\text{O}$ promoted C-prenylation of compound, **2.127** yielded **2.116** in 12% yield (Scheme 2.28). The structure of **2.116** was fully characterised by using ^1H and ^{13}C NMR spectral data.

2.10 STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF ACROVESTONE

2.10.1 Introduction

The cytotoxic activity of acrovestone has been known for a long time. However, no other biological activities of this compound have been reported. The insecticidal properties of acrovestone discovered in this research prompted a detailed analysis of synthetic analogues of acrovestone. This SAR study has the aim of discovering structurally simpler compounds with improved activity.

2.10.2 Structural variations and insecticidal activity of acrovestone

Acrovestone was divided conceptually into six segments (Figure 2.49) and the effects of structural variation in each on insecticidal activity were investigated.

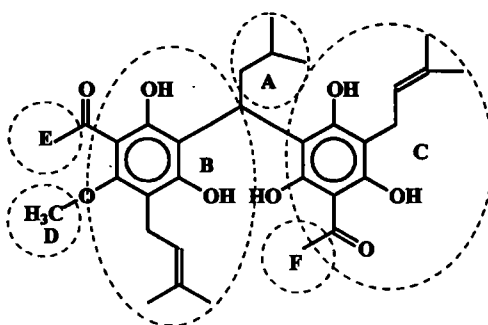


Figure 2.49 : Segmentation of acrovestone

(a) Individual segments

The individual aromatic segments **2.5** (B+D+E-H) and **2.6** (C+F-H) as in natural products, acronylin and demethylacronylin, respectively, are non-insecticidal (Table 2.22) suggesting that activity depends on presence of all the segments of the acrovestone skeleton.

Table 2.22 : Insecticidal activity of structural units and demethylacrovestone

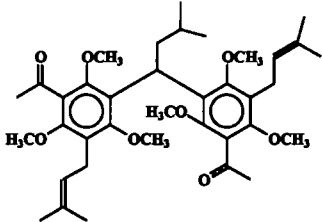
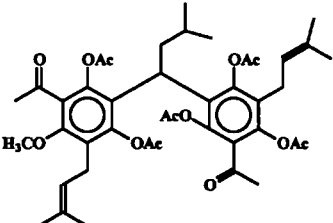
| Compound | Structure | MB | HF |
|------------------------------------|-----------|------|------|
| 1 Acrovestone | | 0.8* | 2.2* |
| 2.10 Demethylacrovestone | | 30 | NT |
| 2.5 | | NT | NT |
| 2.6 | | NT | NT |

Insecticidal activity is given as % mortality (20 µg/insect) or as * LD₅₀ (µg/insect). NT: non toxic. MB : mustard beetle (*Phaedon cochleariae*), HF : housefly (*Musca domestica*).

(b) Hydroxyl groups of acrovestone

Structural modifications of acrovestone were primarily based on two semisynthetic analogues of acrovestone, pentamethoxy acrovestone (2.48) and acrovestone penta-acetate (2.49). Both were non-toxic against mustard beetles and houseflies (Table 2.23 and 2.24) indicating that free hydroxyl groups are necessary for activity.

Table 2.23 : Insecticidal activity of semisynthetic derivatives of acrovestone

| Compound | Structure | MB | HF |
|----------|---|----|----|
| 2.48 |  | NT | NT |
| 2.49 |  | NT | NT |

Insecticidal activity is given as % mortality by topical application (20 µg/insect).

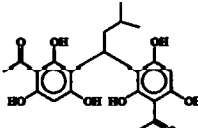
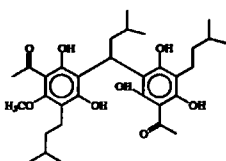
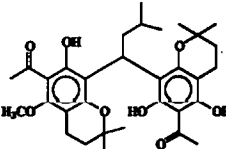
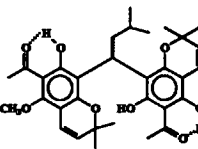
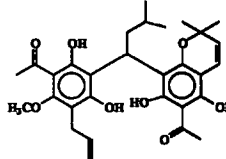
NT: non toxic. MB: mustard beetle (*Phaedon cochleariae*), HF : housefly (*Musca domestica*).

(c) Prenyl groups of acrovestone

Removal of either the prenyl groups or their reduction to afford compounds 2.40 and 2.50, respectively, resulted in total loss of activity. Cyclisation of one

or both prenyl groups results in chroman (2.51) and chromene (2.52 and 2.53) analogues in which free rotation is restricted. They are totally inactive against both mustard beetles and houseflies (Table 2.24) suggesting that the activity strongly depends on free hydroxyls and prenyl groups in the molecule.

Table 2.24 : Insecticidal activity of cyclic analogues of acrovestone

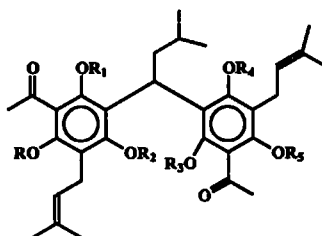
| Compound | Structure | MB | HF |
|----------|---|----|----|
| 2.40 |  | NT | NT |
| 2.50 |  | NT | NT |
| 2.51 |  | NT | NT |
| 2.52 |  | NT | NT |
| 2.53 |  | NT | NT |

Insecticidal activity is given as % mortality by topical application (20 µg/insect). NT: non toxic. MB : mustard beetle (*Phaedon cochleariae*), HF : housefly (*Musca domestica*).

(d) Alkoxy groups of acrovestone

The significance of the methoxy group (D) of acrovestone was recognised because of the poor toxicity of demethylacrovestone (2.10) against both mustard beetles and houseflies (Table 2.22) positioning and number of methoxy groups on the aromatic nucleus were therefore investigated next.

Table 2.25 : Insecticidal activity of methoxy analogues of acrovestone



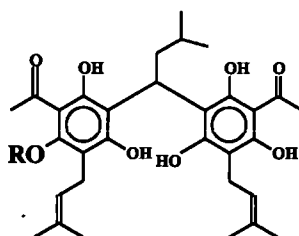
| Compound | R, | R ₁ , | R ₂ , | R ₃ , | R ₄ , | R ₅ | MB |
|----------|-----------------|------------------|------------------|------------------|------------------|-----------------|-----|
| 1 | CH ₃ | H | H | H | H | H | 100 |
| 2.54 | H | CH ₃ | H | H | H | H | 65 |
| 2.55 | H | H | CH ₃ | H | H | H | 85 |
| 2.56 | CH ₃ | H | H | H | H | CH ₃ | 85 |
| 2.57 | CH ₃ | CH ₃ | H | H | H | H | 20 |
| 2.58 | CH ₃ | H | CH ₃ | H | H | H | 30 |
| 2.59 | H | CH ₃ | CH ₃ | H | H | H | 20 |
| 2.48 | CH ₃ | CH ₃ | CH ₃ | CH ₃ | CH ₃ | CH ₃ | NT |

Insecticidal activity is given as % mortality by topical application (20 µg/insect). NT : non toxic. MB : mustard beetle (*Phaedon cochleariae*).

Moving the methoxy group within the same ring gives compounds, **2.54** and **2.55** with slightly decreased activity (Table 2.25). This suggests that the level of activity is sensitive to the position of the methoxy group within the ring. Introduction of a second methoxy group to the same ring (**2.57-2.59**) significantly reduced the activity. Interestingly, introduction of a second methoxy group to the other ring gives the symmetrical molecule, **2.56** when activity is mostly retained (Table 2.25). Addition of more methoxy groups (**2.48**) completely removed the activity (Table 2.25). These bioassay data suggested that activity is closely linked with the number of methoxy groups and in particular their position in the molecule.

Further investigation of the nature of the alkoxy substituents (Table 2.26) clearly demonstrated that compounds with more bulky alkoxy groups have no significant activity compared to acrovestone (Table 2.22 and 2.26), thus indicating strict steric requirements in this region of the acrovestone molecule for insecticidal activity. Methoxy remains the best alkoxy group for activity.

Table 2.26 : Insecticidal activity of alkoxy analogues of acrovestone



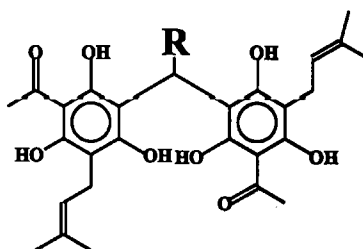
| Compound | R | MB | HF |
|----------|---|----|----|
| 2.77 | | 10 | NT |
| 2.78 | | 15 | 30 |
| 2.79 | | 05 | 43 |
| 2.80 | | NT | NT |

Insecticidal activity is given as % mortality by topical application (20 µg/insect). NT: non toxic, MB : mustard beetle (*Phaedon cochleariae*), HF : housefly (*Musca domestica*).

(e) Central alkyl group of demethylacrovestone

The SAR study was next extended to compounds in which the central alkyl group (R) was varied using demethylacrovestone (2.10) as the model compound. Bioassay indicated that compounds with central methyl (2.82) and isopropyl (2.85) groups were much more active than demethylacrovestone (Table 2.22 and 2.27). However, a relatively low level of activity was observed for compounds 2.83 and 2.84.

Table 2.27 : Insecticidal activity of analogues of demethylacrovestone



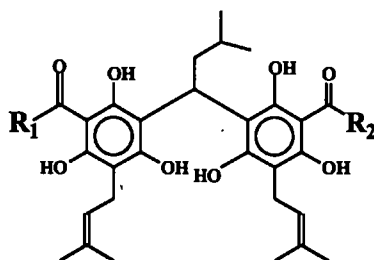
| Compound | R | MB | HF |
|----------|-----------------|----|----|
| 2.81 | H | NT | NT |
| 2.82 | Me | 60 | NT |
| 2.83 | Et | 15 | NT |
| 2.84 | Pr | 20 | NT |
| 2.85 | ⁱ Pr | 55 | NT |
| 2.10 | ⁱ Bu | 30 | NT |







Insecticidal activity is given as % mortality by topical application (20 µg/insect). NT: non toxic. MB : mustard beetle (*Phaedon cochleariae*), HF : housefly (*Musca domestica*).

(f) Acyl groups of demethylacrovestone

Replacement of one or both methyl groups (E and F, Figure 2.49) with an isopropyl or isobutyl groups resulted in total loss of insecticidal activity, indicating that, as with region D, there are strict steric requirements in this region of acrovestone molecule.

Table 2.28 : Insecticidal activity of demethylacrovestone analogues with different acyl groups.



| Compound No | R ₁ | R ₂ | MB |
|-------------|---|---|----|
| 2.95 |  | CH ₃ | NT |
| 2.96 |  |  | NT |
| 2.97 |  | CH ₃ | NT |
| 2.98 |  |  | NT |

Insecticidal activity is given as % mortality by topical application (20 µg/insect). NT: non toxic. MB : mustard beetle (*Phaedon cochleariae*).

(g) Summary

SAR studies indicate that all the component moieties (A to F) are necessary, and that the number, position and size of substituents are critical for insecticidal activity. Optimum activity appears to be associated with the presence of a single methoxy group. The only region amenable to variations, leading to increased activity is the bridgehead group (Table 2.27).

2.10.3 Hybrid compounds

Replacement of the methyl group in the acyl moiety (Table 2.28) with an isobutyl or isopropyl groups resulted in loss of activity, in contrast to other natural products, where these groups confer insecticidal activity. Kunzein 0 (2.101 and 2.102), Table 2.28) are particularly interesting examples because of their overall structural resemblance to acrovestone. They too have an alkylmethyl bridgehead group and a basic phloroglucinol moiety, but they do not have prenyl groups. The syncarpic acid moiety (2.108) present in the kunzeins¹⁷³ is biosynthetically related to phloroglucinol, so was considered as an alternative group in the SAR study. All four 'hybrid' compounds, derived from combinations of acrovestone and kunzein 0 structures (Table 2.29), were synthesised and tested. Hybrid compounds 2.103 and 2.104, which incorporate syncarpic acid moiety, were less active than corresponding pair (2.105 and 2.106) containing only phloroglucinol moieties. Overall activity of latter compounds is higher than that of acrovestone and kunzeins. In comparison with acrovestone, the most notable features of these compounds are the absence of a prenyl group (considered essential for activity of acrovestone, see section (c)) and the presence of an isopropyl group. As in acrovestone series, the presence of a single methoxy group in both series resulted in increased activity. In contrast to the conclusions from the systematic SAR study on acrovestone (Section (a) to (g)), this part of the study (with hybrid compounds)

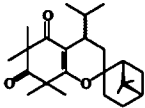
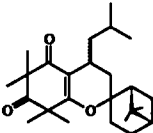
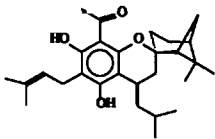
Table 2.29 : Insecticidal activity of acrovestone-kunzein hybrid compounds

| Compound | Structure | MB | HF |
|--------------------------|-----------|-------|------------------------|
| Kunzein 0 (2.101) | | 0.5* | 56% (0.2 µg/insect) |
| Kunzein 1 (2.102) | | 3.5* | 2.0* |
| Acrovestone (1) | | 0.8* | 2.2* |
| 2.103 | | 5% | NT |
| 2.104 | | 85% | NT |
| 2.105 | | 0.51* | 1.55* |
| 2.106 | | 0.7* | 0.8* |

Insecticidal activity is given as % mortality (20 µg/insect) or as * LD₅₀ (µg/insect) by topical application. NT: non toxic. MB : mustard beetle (*Phaedon cochleariae*), HF : housefly (*Musca domestica*).

indicated that substantial variations are tolerated in one segment of acrovestone and that SARs are not additive. A similar contrast in activities of related but conformationally restricted compounds in kunzein series is also apparent from a previous study¹⁷³. The natural product **2.114a** containing an isopropyl bridge group exhibited low insecticidal activity but surprisingly compound **2.114b** containing the isobutyl group (as in acrovestone) showed none¹⁷³. These observations prompted the investigation of conformationally restricted analogues in which the syncarpic acid moiety has been replaced by a phloroglucinol moiety, a substitution shown to increase insecticidal activity. In the time available, only one such analogue (**2.116**) could be synthesised and it proved to be nontoxic to insects (Table 2.30).

Table 2.30 : Insecticidal activity of acrovestone-robustadial compounds

| Compound | Structure | MB | HF |
|---------------|---|----|----|
| 2.114a |  | 12 | 19 |
| 2.114b |  | NT | NT |
| 2.116 |  | NT | NT |

Insecticidal activity is given as LD₅₀ (µg/insect) by topical application. NT: non toxic.

MB : mustard beetle (*Phaedon cochleariae*), HF : housefly (*Musca domestica*).

2.11 MODE OF ACTION OF ACROVESTONE

The recognition of the mode of action of acrovestone is important for further SAR studies. The slow death of mustard beetles treated with acrovestone arbitrarily suggested that it is not a nerve toxin (which often effect rapid death) but could possibly be a respiratory inhibitor. Therefore an *in vitro* mitochondrial complex III assay has been carried out and showed that acrovestone inhibits mitochondrial respiration at a low concentration giving an IC_{50} value of 4×10^{-8} M. The inhibitory activity is comparable with recently developed plant derived naphthoquinones¹⁸³.

The poor *in vivo* (LD_{50} 0.8 and 2.2 μ g/insect, Table 2.22 in page138) and strong *in vitro* activity ($IC_{50} = 4 \times 10^{-8}$ M) of acrovestone suggests that the molecule is susceptible to metabolic attack before it reaches the target site.

2.12 OTHER BIOLOGICAL ACTIVITIES OF ACROVESTONE AND ANALOGUES

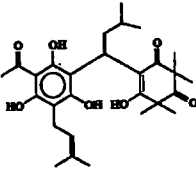
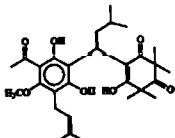
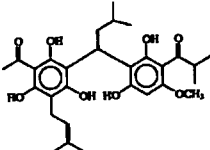
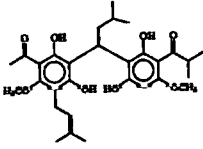
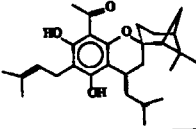
In addition to the insecticidal assays, brine shrimp lethality, antibacterial, and antifungal assays were carried out with acrovestone and its analogues. Acrovestone and analogues were non-toxic against a series of fungal species, *Rhizoctonia solani*, *Gaeummanomyces graminis*, *Colletotrichum coccodes*, *Fusarium sulphareum* and *Botrytis cinera*.

(a) Activity against brine shrimp larvae

Activities of the hybrid and reference compounds are shown in Table 2.32.

The contrast between methoxy and hydroxy analogues (1, 2.104-2.106 and 2.10, 2.103 and 2.116) is much greater for brine shrimp (*Artimia salina*

Table 2.32 : Brine Shrimp Lethality assay of acrovestone analogues

| Compound | Structure | Activity |
|----------------------------|--|----------|
| acrovestone (1) | | 0.2 |
| demethylacrovestone (2.10) | | NT |
| 2.103 |  | NT |
| 2.104 |  | 0.2 |
| 2.105 |  | 0.3 |
| 2.106 |  | 0.2 |
| 2.116 |  | NT |

Brine Shrimp Lethality activity is given as LC₅₀ (ppm). NT: non toxic.

Crustacia) than for Mustard beetles (Arthropoda), except in hybrid series (2.103 and 2.97). Compound 2.116 was found to be nontoxic against brine shrimp larvae.

(b) Antibacterial activity

Results for limited number of compounds tested against six bacterium species by collaborators at the Jodrell Laboratory, Kew are shown in Table 2.31. Although large variations in activity are apparent, no parallels can be drawn between chemical structures or their insecticidal activity. For example, acrovestone associated with increased insecticidal activity, is less toxic than demethylacrovestone and compounds with insignificant insecticidal activity (2.10, 2.50, 2.54) exhibit relatively high antibacterial activity.

Table 2.31 : Antibacterial activity of acrovestone analogues

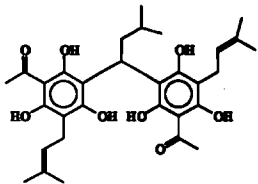
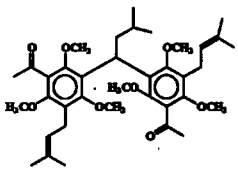
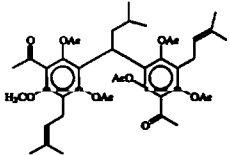
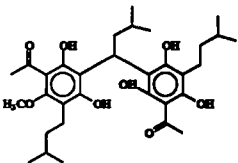
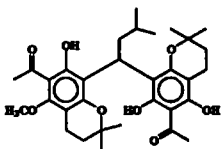
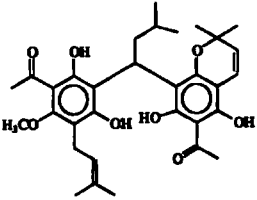
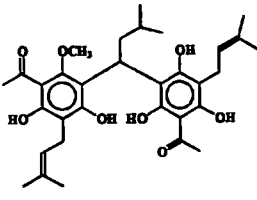
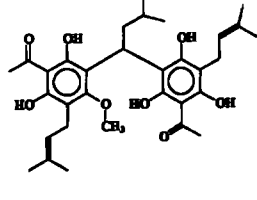
| Compound | BS | EC | FS | PV | SA | SF |
|---|----|----|-----|-----|-----|-----|
| acrovestone (1) | 9 | 0 | 9 | 19 | 0 | 0 |
|  (2.10) | 11 | 0 | 27* | 28* | 28* | 21 |
|  (2.48) | 10 | 0 | 8 | 0 | 0 | 9 |
|  (2.49) | 9 | 0 | 19 | 20 | 21 | 17 |
|  (2.50) | 10 | 0 | 27* | 31* | 27* | 27* |
|  (2.51) | 8 | 0 | 8 | 8 | 9 | 8 |

Table contd.

| | | | | | | |
|---|----|----|-----|-----|-----|----|
|  (2.53) | 8 | 0 | 1 | 20 | 21 | 19 |
|  (2.54) | 8 | 0 | 28* | 28* | 29* | 21 |
|  (2.55) | 2 | 0 | 0 | 9 | 9 | 1 |
| (+) Control | 39 | 28 | 28 | 28 | 19 | 19 |
| (-) Control | 10 | 2 | 6 | 2 | 2 | 2 |

Antibacterial activity is given as mean value of inhibitory zone in mm at 100 ppm dose.

Chloramphenicol at 1ppm level was used as (+) control. * Significantly more active than no-treatment control (t-test, $P < 0.01$).

BS: *Bacillus subtilis*, EC: *Escherichia coli* K-12, FS: *Flavobacterium suaveolens*, PV: *Proteus vulgaris*, SA: *Staphylococcus aureus*, SF: *Streptococcus faecalis*.

2.13 OVERALL CONCLUSION

This study has demonstrated the advantage of combining a conventional SAR approaches based on synthetic analogues, typically incorporating small but systematic structural changes, with one using larger changes through synthesis of hybrid compounds from related natural products. Extending the SAR study to other species to establish a spectrum of activity was limited by time but the brine shrimp and antibacterial data gave an indication of differences.

CHAPTER 3

EXPERIMENTAL

3.1 GENERAL EXPERIMENTAL

3.1.1 Collection and preparation of plant material

Various parts of all plants were collected from different geographical locations in Sri Lanka during the period of 1994-1997. Stem bark, leaves and fruits of *Acronychia pedunculata* were collected from Western Province, Colombo. Stem bark and leaves of *Pleurostyliia opposita*, *Alseodaphne semicarpifolia* and *Walsura piscidia* were collected from Central Province, Anuradhapura.

The plants were identified by the late Professor S. Balasubramaniam of the Department of Botany, University of Peradeniya, Sri Lanka. The voucher specimens of plants were deposited in the Department of Botany, University of Colombo, Sri Lanka. The plant materials were shade dried, ground and stored at 10 °C until required for extraction.

3.1.2 Extraction

Small scale extractions were carried out by stirring finely powdered plant material (20 g) in appropriate solvents for 20 min at room temperature. The extraction was sequential according to the ascending order of the polarity of petroleum ether, ethyl acetate and ethanol (2 × 100 ml, each). Large scale extractions were performed by stirring plant material in appropriate solvents for several days or using a microwave extraction procedure^{184, 185}. Each extract was decanted, filtered through a celite bed and concentrated *in vacuo*.

The yields of the compounds obtained were expressed as a percentage of the weight of dry plant material used for extraction.

3.1.3 Chromatography

Thin layer chromatography (tlc) systems were extensively used to monitor the progress of all chromatographic separations. For this either plastic-backed plates supporting a 0.25 mm layer of silica gel 60F₂₅₄ (No. 5735), or pre-coated glass plates supporting a 0.25 mm layer of RP-18F₂₅₄ (No. 15685) and silica gel 60F₂₅₄ (No. 5719), or pre-coated HPTLC glass plates supporting a 0.10 mm layer of silica gel 60F₂₅₄ (No. 5628) were used.

Anisaldehyde-H₂SO₄, 5% phosphomolybdic acid-ethanol, Dragendorff's reagent¹²⁸ and UV light (λ = 254 and 366 nm) were used to visualise the compounds on tlc plates.

Dry column flash^{186, 187}, flash column¹⁸⁸, column¹⁸⁹ and preparative thin layer chromatographic¹⁸⁹ techniques were widely used for the separation of natural products and synthetic compounds. Silica gel 60H supplied by Merck (No. 64271) was used for dry column chromatography. Flash column chromatography was carried out using 230-400 mesh silica gel (particle size: 40-63 μ m), supplied by Merck (No. 9385). Silica gel supplied by Merck [(No. 7734) 70-230 mesh ASTM, particle size: 63-200 μ m] was used for gravity column chromatography. Reverse phase gravity column chromatography and flash column chromatography was carried out using silica gel 60 RP-18 (particle

size: 40-63 μ m), supplied by Merck (No. 10167). Preparative tlc was carried out using 20 \times 20 cm glass plates supporting a 1 mm layer of silica gel 60GF, supplied by Anachem (No. 2013) and products were best visualised by UV light.

Normal phase semi-preparative and preparative HPLC systems were used in the final purification of compounds. HPLC grade solvents supplied by Rathburn were used in HPLC separations and degassed using an ultrasonic bath for about 30 min before use. Semi-preparative HPLC was performed with 10 mm ID \times 250 mm L Dynamax, 60 Å, 8 μ silica column coupled to Gilson Model 305 and 306 LC pumps and an Applied Biosystems 1000s Diode array detector, monitored at four different wavelengths simultaneously. Preparative HPLC separations were performed with 21.44 mm ID \times 250 mm L Dynamax, 60 Å, 8 μ silica column coupled to Gilson Model 305 and 306 LC pumps and a Dynamax, UV-1, variable wavelength UV/visible absorbance detector.

3.1.4 Spectroscopy

A Jeol JNM-GX 400 FT NMR spectrometer, operating at 400 MHz was used to record ^1H NMR spectra, and ^{13}C NMR spectra were recorded on the same instrument, operating at 100 MHz. The DEPT, COSY and NOESY spectra were recorded at 400 MHz on a Jeol JNM-GX 400 FT NMR spectrometer. DEPT experiments were recorded at 90° and 135° and NOESY spectra were recorded with mixing time of 500 ms. Deuteriochloroform (CDCl_3), deuteriated acetone (CD_3COCD_3) or deuteriated methanol (CD_3OD) was used as a solvent

for NMR and chemical shift values (δ) were reported in ppm downfield from tetramethylsilane (TMS).

High resolution mass spectra were recorded on a Micromass Autospec double focusing mass spectrometer equipped with an electrospray API interface. Low resolution mass measurements were recorded on Autospec mass spectrometer at 70 eV.

Infrared spectra were recorded using a Nicolet Impact 410 IR and FT IR spectrometer with the compounds as a solution in chloroform or as KBr discs. UV spectra were recorded in ethanol using a Shimadzu UV-160A UV-Visible spectrophotometer.

Optical rotation was recorded, using a known concentration of compound in chloroform, on a Thorn NPL143 polarimeter. Melting points were recorded using a Gallenkamp electrothermal capillary tube melting point apparatus and are uncorrected.

3.1.5 Purification of chemicals

THF was distilled from sodium-benzophenone and stored under N₂. Petroleum ether and diethyl ether were dried by storing over sodium wire prior to use. All solvents and reagents used were analytical grade.

The purity of all starting materials of reactions was confirmed by recording a ^1H NMR spectrum. Aldehydes were distilled and stored under N_2 prior to use.

3.1.6 Insecticidal bioassay

Insecticidal activity of plant extracts, fractions and compounds was assessed against mustard beetles, houseflies, whitefly, diamondback moth larvae and mites. A 2% (w/v) solution of the sample was prepared by using either an acetone or acetone:ethanol (1:1) solution and this was used as a stock solution for bioassay. Plant extracts were tested at much larger doses in preliminary assays, 20 $\mu\text{g}/\text{insect}$ for mustard beetle and housefly, 10 $\mu\text{g}/\text{insect}$ for diamondback moth, 2000 ppm/insect for whitefly and 1000 ppm/insect for mites. For line tests a closely spaced range of concentrations was used in comparative tests with the standard bioresmethrin. LD_{50} and LC_{50} values were calculated from the several mortalities, using a probit analysis software package (POLO-PC)¹⁹⁰.

Bioassay guided fractionation of natural products was carried out using mustard beetles as a test insect species. Insecticidal bioassays against houseflies, whitefly, diamondback moth larvae and mites¹⁹¹ were carried out by colleagues in the Resistance group within the Biological and Ecological Chemistry Department at IACR-Rothamsted and procedures are described in Appendix 1.

(a) Mustard beetle (*Phaedon cochleariae* Fab)

The adult mustard beetles were treated with a solution of the compound of interest by topical application. A 1 μ l dose of the test solution was applied ventrally to mustard beetles (20 per concentration) using an Arnold micro drop applicator. The treated beetles were stored in petri dishes at 20 °C, and assessed for mortality after 48 h. Line tests used 5 dose levels per compound and three replicates of 20 beetles for each.

3.1.7 Brine shrimp lethality bioassay (BST)

Artemia salina Leach larvae were cultured as described in literature^{192, 193}. Vials were filled with 10 ml of brine and 20 larvae. Test compounds (10 mg) were dissolved in ethanol (1 ml) and 1, 0.5, 0.4, 0.3, 0.2 and 0.1 μ l of the test solution was added to the vials containing brine and larvae. Controls were prepared by adding 1 μ l of ethanol to 10 ml of brine containing 20 larvae. Each treatment was triplicated and vials were kept randomly under continuous light at room temperature. The percentage of survivors were taken after 24 h and LC₅₀ values were calculated using probit analysis software package (POLO-PC)¹⁹⁰.

3.1.8 Fungicidal bioassay

Fungicidal bioassays against *Rhizoctonia solani*, *Gaeummanomyces graminis*, *Colletotrichum coccodes*, *Fusarium sulphareum* and *Botrytis cinerea* were carried out by colleagues in Entomology and Nematology Department at IACR-Rothamsted.

3.1.9 Antibacterial bioassay

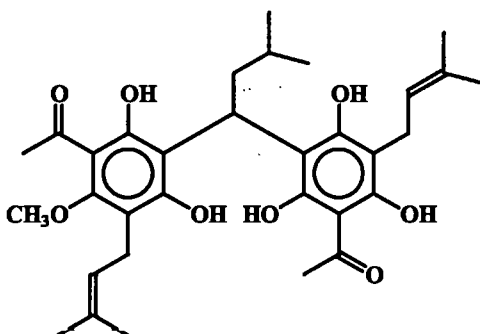
Antibacterial bioassay against *Bacillus subtilis* (+), *Escherichia coli* K-12 (-), *Flavobacterium suaveolens* (-), *Proteus vulgaris* (-), *Staphylococcus aureus* (+) and *Streptococcus faecalis* (+) were done at Royal Botanical gardens, Kew. The procedures are described in Appendix 1.

3.1.10 Mode of action studies

In vitro mitochondrial complex III assay was carried out by colleague at Biological and Ecological Chemistry Department at IACR-Rothamsted.

3.2 EXTRACTION AND ISOLATION OF INSECTICIDAL COMPOUNDS FROM *ACRONYCHIA PEDUNCULATA*

3.2.1 Isolation of compound 1



Air-dried, finely powdered stem bark of *Acronychia pedunculata* (100 g) was exclusively extracted with petroleum ether (3×300 ml) at room temperature, using the method described in section 3.1.2. The combined extracts were concentrated *in vacuo* to afford a yellow gummy residue (5.0 g).

The petroleum ether extract (4.5 g) was subjected to dry column flash chromatography on silica gel using petroleum ether:diethyl ether (7:3, v/v) to obtain the insecticidal fraction as a yellow solid. The recrystallisation of this solid in methanol afforded insecticidal compound 1 as a bright yellow crystals (400 mg, 0.4%, m.p. 142-144 °C).

ν_{\max} (CHCl_3 , cm^{-1}); 3260, 2959, 2920, 2865, 1608, 1455, 1365, 1320. λ_{\max} (EtOH, nm); 230, 296, 339.

δ_{H} (CDCl_3); 0.84 (6H, br s, $2 \times \text{CH}_3$), 1.41 (1H, m, CH), 1.68 (3H, s, CH_3), 1.75 (3H, s, CH_3), 1.77 (3H, s, CH_3), 1.82 (3H, s, CH_3), 2.24 (2H, m, CH_2),

2.67 (3H, s, CH₃), 2.71 (3H, s, CH₃), 3.32 (2H, d, *J* 6.6, CH₂), 3.34 (2H, d, *J* 6.6, CH₂), 3.71 (3H, s, OCH₃), 4.74 (1H, t, *J* 7.7, CH), 5.21 (2H, t, *J* 6.6, 2 × CH), 6.50 (1H, br s, OH), 9.30 (1H, br s, OH), 10.20 (1H, br s, OH), 15.58, (1H, s, OH), 15.70 (1H, br s, OH).

δ_C (CDCl₃); 17.9 (CH₃), 18.0 (CH₃), 22.3 (CH₂), 22.5 (CH₃), 22.7 (CH₃), 23.2 (CH₂), 25.6 (CH₃), 25.7 (CH₃), 27.2 (CH), 28.8 (CH), 30.6 (CH₃), 32.6 (CH₃), 39.6 (CH₂), 62.6 (OCH₃), 104.9 (C_q), 106.3 (C_q), 108.3 (C_q), 108.6 (C_q), 113.2 (C_q), 116.8 (C_q), 121.6 (CH), 123.2 (CH), 131.6 (C_q), 136.7 (C_q), 158.3 (C_q), 158.4 (C_q), 160.3 (C_q), 160.8 (C_q), 160.9 (C_q), 162.7 (C_q), 204.2 (C_q), 204.3 (C_q). Mass Spec. (EI) *m/z* 554 M⁺.

3.3 EXTRACTION, ISOLATION AND STRUCTURAL STUDIES OF COMPOUNDS FROM *PLEUROSTYLLIA OPPOSITA*

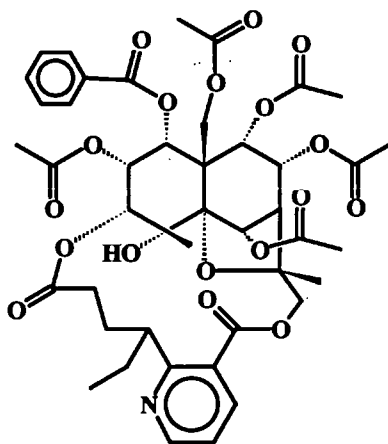
3.3.1 Isolation of compounds 2, 3 and 4

The shade-dried, finely powdered stem bark of *Pleurostyllia opposita* (500 g) was extracted with ethyl acetate (2 × 1.5 l) using the microwave extraction procedure. The extract was concentrated *in vacuo* to obtain a gummy material (15 g). This (14.5 g) was subjected to dry column flash chromatography on silica gel eluting with a gradient solvent system of petroleum ether:ethyl acetate (2:3, 3:7, 1:4, 1:9, 2 × 75 ml, each). The insecticidal fraction (1.3 g) was further purified by silica gel flash column chromatography eluting with

petroleum ether:ethyl acetate:isopropanol (6:3:1 to 5:4:1; 200 ml, 400 ml, respectively), to afford the active fraction (135.0 mg).

The active fraction (135 mg) was subjected to preparative HPLC (silica column, 10 ml min⁻¹, UV 254 nm) eluting with hexane:ethyl acetate:isopropanol (5:4:1) to obtain insecticidal compounds **2** (38 mg, 0.03%, retention time 6.0 min) and **3** (22 mg, 0.02%, retention time 7.2 min) and non-insecticidal compound **4** (7.4 mg, 0.01%, retention time 14.0 min) as yellow oils.

Compound 2



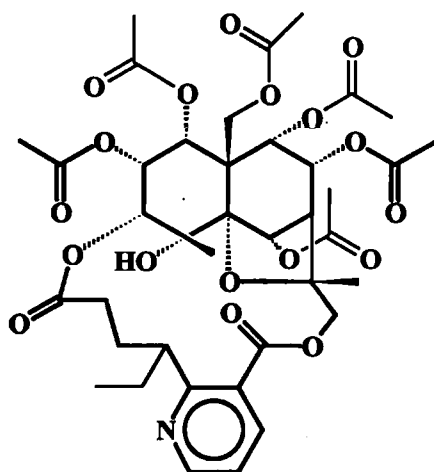
ν_{\max} (CHCl₃, cm⁻¹); 3689, 3468, 3436, 3020, 2985, 1738, 1602, 1467, 1378, 1247, 1044, 737, 733. λ_{\max} (EtOH, nm); 269, 229, 203.

δ_{H} (CDCl₃); δ 0.70 (3H, t, J 7.3, CH₃), 1.40 (3H, s, CH₃), 1.60 (3H, s, CH₃), 1.75 (3H, s, CH₃), 1.75-2.00 (2H, m, CH₂), 1.95-2.20 (2H, m, CH₂), 2.12 (1H, m, CH), 2.25 (3H, s, CH₃), 2.30 (3H, s, CH₃), 2.35 (3H, s, CH₃), 2.25-2.35

(2H, m, CH₂), 2.40 (3H, s, CH₃), 3.60 (1H, d, *J* 11.9, CH₂), 4.40 (1H, m, CH), 4.60 (1H, d, *J* 13.4, CH), 4.90 (1H, br s, OH), 5.00 (1H, d, *J* 2.4, CH), 5.20 (1H, dd, *J* 4.0, 2.4, CH), 5.34 (1H, d, *J* 13.4, CH), 5.38 (1H, d, *J* 5.8, CH), 5.55 (1H, m, CH), 5.85 (1H, d, *J* 4.0, CH), 6.00 (1H, d, *J* 11.9, CH₂), 7.00 (1H, s, CH), 7.30 (1H, dd, *J* 7.9, 4.6, CH), 7.42 (2H, dd, *J*₁ = *J*₂ 7.6, CH), 7.54 (1H, dd, *J*₁ = *J*₂ 7.6, CH), 7.83 (1H, d, *J* 7.0, CH), 8.83 (1H, dd, *J* 4.6, 1.8, CH), 8.40 (1H, dd, *J* 7.9, 1.8, CH).

δ_C (CDCl₃); δ 12.0 (CH₃), 18.0 (CH₃), 19.9 (CH₃), 20.9 (CH₃), 21.0 (CH₃), 21.4 (CH₃), 21.6 (CH₃), 22.8 (CH₃), 28.8 (CH₂), 31.2 (CH₂), 31.8 (CH₂), 42.1 (CH), 50.7 (CH), 52.4 (C_q), 60.0 (CH₂), 68.8 (CH), 69.8 (CH), 69.8 (C_q), 69.9 (CH₂), 70.0 (CH), 71.4 (CH), 73.6 (CH₂), 75.7 (CH), 84.9 (C_q), 94.0 (C_q), 120.7 (CH), 125.2 (C_q), 128.5 (2 × CH), 129.4 (C_q), 129.4 (CH), 129.5 (CH), 135.5 (CH), 138.1 (CH), 153.8 (CH), 164.6 (C_q), 166.4 (C_q), 168.5 (2 × C_q), 168.9 (C_q), 169.9 (C_q), 170.0 (C_q), 170.4 (C_q), 172.2 (C_q). Mass Spec. (ESI) 882.3192. C₄₄H₅₂O₁₈N requires 882.184.

Compound 3



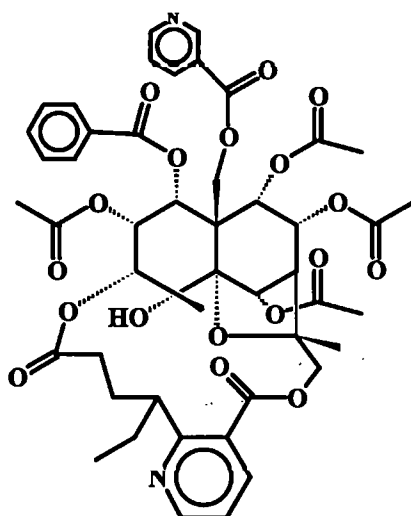
ν_{\max} (CHCl₃, cm⁻¹); 3617, 3542, 3462, 3029, 2973, 2869, 2257, 2092, 1885, 1740, 1565, 1377, 1033, 920, 845; λ_{\max} (EtOH, nm) ; 269, 206.

δ_{H} (CDCl₃); δ 0.70 (3H, t, J 7.3, CH₃), 1.30 (3H, s, CH₃), 1.68 (3H, s, CH₃), 1.73 (3H, s, CH₃), 1.90 (3H, s, CH₃), 1.75-2.00 (2H, m, CH₂), 2.12 (1H, m, CH), 2.15 (6H, s, 2 \times CH₃), 2.20 (3H, s, CH₃), 1.95-2.20 (4H, m, 2 \times CH₂), 2.35 (3H, s, CH₃), 2.25-2.35 (2H, m, CH₂), 3.60 (1H, d, J 11.7, CH₂), 4.40 (1H, m, CH), 4.50 (1H, d, J 13.5, CH₂), 4.90 (1H, br s, OH), 4.97 (1H, d, J 2.4, CH), 5.15 (1H, d, J 13.5, CH₂), 5.20 (1H, dd, J 3.9, 2.4, CH), 5.48 (1H, d, J 5.8, CH), 5.55 (1H, m, CH), 5.55 (1H, d, J 3.9, CH), 6.00 (1H, d, J 11.7, CH₂), 7.00 (1H, s, CH), 7.13 (1H, dd, J 7.9, 4.6, CH), 8.40 (1H, dd, J 7.9, 1.8, CH), 8.83 (1H, dd, J 4.6, 1.8, CH).

δ_{C} (CDCl₃); δ 11.9 (CH₃), 18.0 (CH₃), 20.5 (CH₃), 20.8 (CH₃), 21.0 (CH₃), 21.3 (CH₃), 21.6 (CH₃), 22.9 (CH₃), 25.3 (CH₃), 28.8 (CH₂), 31.3 (CH₂), 31.8 (CH₂), 42.2 (CH), 50.8 (CH), 52.1 (C_q), 60.0 (CH), 69.6 (C_q), 69.7 (CH), 69.9

(CH₂), 70.8 (CH), 73.4 (CH), 73.6 (CH), 75.7 (CH), 84.7 (C_q), 93.9 (C_q), 120.8 (CH), 129.6 (C_q), 138.2 (CH), 153.8 (CH), 166.4 (C_q), 168.0 (2 × C_q), 168.8 (C_q), 169.1 (C_q), 169.9 (C_q), 170.0 (C_q), 170.1 (C_q), 170.3 (C_q), 172.3 (C_q). Mass Spec. (ESI) *m/z* 820.3028 [M+H]⁺, 842.2847 [M+Na]⁺, 858.2587 [M+K]⁺ (Found : [M+H]⁺, 820.3035. C₃₉H₅₀O₁₈N requires 820.3027).

Compound 4



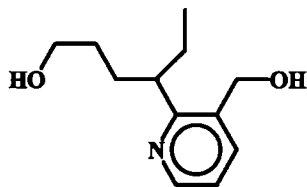
ν_{\max} (CHCl₃, cm⁻¹) ; 3728, 3448, 3021, 2962, 2927, 1751, 1603, 1567, 1456, 1372, 1235, 1098, 1039, 920, 870, 740. λ_{\max} (EtOH, nm) ; 268, 228, 225, 205. δ_{H} (CDCl₃); 0.72 (3H, t, *J* 7.3, CH₃), 1.35 (3H, s, CH₃), 1.65 (3H, s, CH₃), 1.75 (3H, s, CH₃), 1.75-2.00 (2H, m, CH₂), 1.95-2.20 (2H, m, CH₂), 2.12 (1H, m, CH₂), 2.14 (3H, s, CH₃), 2.23 (3H, s, CH₃), 2.34 (3H, s, CH₃), 2.25-2.35 (2H, m, CH₂), 3.40 (1H, d *J* 11.9, CH₂), 4.40 (1H, m, CH), 4.55 (1H, d, *J* 13.4, CH₂), 4.90 (1H, br s, OH), 5.15 (1H, d, *J* 2.4, CH), 5.20 (1H, dd, *J* 4.0, 2.4, CH), 5.45 (1H, d, *J* 13.4, CH₂), 5.55 (1H, m, CH), 5.65 (1H, d, *J* 5.8, CH),

6.05 (1H, d, J 4.0, CH₂), 6.05 (1H, d, J 11.9, CH₂), 7.06 (1H, s, CH), 7.30 (2H, dd, $J_1 = J_2$ 7.6, CH), 7.40 (1H, dd, J 7.9, 4.6, CH), 7.50 (1H, m, CH), 7.54 (1H, dd, $J_1 = J_2$ 7.6, CH), 7.75 (2H, d, J 7.0, CH), 8.40 (1H, m, CH), 8.50 (1H, dd, J 7.9, 1.8, CH), 8.90 (1H, br s, CH), 8.90 (1H, dd, J 4.6, 1.8, CH), 9.30 (1H, br s, CH).

δ_C (CDCl₃); δ 12.0 (CH₃), 18.1 (CH₃), 19.9 (CH₃), 20.9 (CH₃), 21.3 (CH₃), 21.7 (CH₃), 23.2 (CH₃), 28.7 (CH₂), 31.2 (CH₂), 31.8 (CH₂), 42.2 (CH), 50.8 (CH), 52.4 (C_q), 60.3 (CH₂), 68.9 (CH), 69.9 (C_q), 70.3 (CH₂), 71.2 (2 \times CH), 73.6 (CH), 73.9 (CH), 75.5 (CH), 85.1 (C_q), 94.0 (C_q), 121.0 (CH), 123.8 (C_q), 124.8 (CH), 124.9 (C_q), 128.6 (C_q), 128.6 (2 \times CH), 129.5 (2 \times CH), 133.6 (CH), 137.6 (CH), 138.6 (CH), 150.8 (CH), 153.5 (CH), 153.9 (CH), 163.4 (C_q), 164.6 (C_q), 166.1 (C_q), 167.9 (C_q), 168.4 (C_q), 168.9 (C_q), 170.0 (2 \times C_q), 172.2 (C_q). Mass Spec. (ESI) m/z 945.3293 [M+H], 967.3113 [M+Na]⁺, 983.2552 [M+K]⁺ (Found : [M+H]⁺, 945.3294. C₄₈H₅₃O₁₈N₂ requires 945.32925).

3.3.2 Structural studies of compound 2

(a) Lithium aluminium hydride reduction¹²⁵



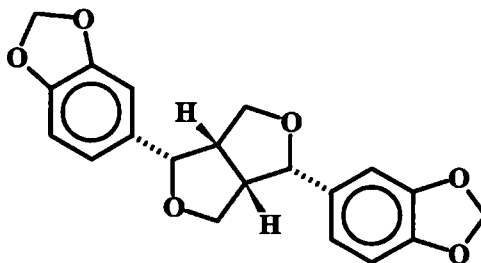
A solution of compound 2 (35 mg) in dry THF (5 ml) was added to a stirring solution of LiAlH_4 (50 mg) in dry THF (10 ml) at 0°C under N_2 . The mixture was gradually warmed to room temperature and stirred for 12 h. The reaction mixture was treated with successive dropwise addition of water (0.4 ml), 15% NaOH (0.4 ml) and water (1.2 ml). The resultant white precipitate was filtered and washed with ethyl acetate (3×10 ml). The filtrate and washings were concentrated *in vacuo* to obtain the fragment 1 as an oil (10 mg, 28.5 %).

δ_{H} (CDCl_3); 0.74 (3H, t, J 7.3, CH_3), 1.92 (2H, m, CH_2), 1.93 (2H, m, CH_2), 2.01 (2H, m, CH_2), 2.30-2.50 (2H, br s, $2 \times \text{OH}$), 3.00 (1H, m, CH), 3.71 (1H, t, J 5.8, CH_2), 4.65 (1H, d, J 12.8, CH_2), 4.81 (1H, d, J , 12.8, CH_2), 7.10 (1H, dd, J 7.9, 4.8, CH), 7.69 (1H, dd, J 7.9, 1.8, CH), 8.52 (1H, d, J 4.8, CH).

δ_{C} (CDCl_3); 12.1 (CH_3), 28.6 (CH_2), 30.2 (CH_2), 31.2 (CH_2), 43.0 (CH), 62.1 (CH_2), 63.0 (CH_2), 120.9 (CH), 134.4 (C_q), 135.7 (CH), 148.5 (CH), 162.8 (C_q).

3.4 EXTRACTION AND ISOLATION OF INSECTICIDAL COMPOUNDS FROM *ALSEODAPHNE SEMICARPIFOLIA*

3.4.1 Isolation of compound 5



The shade-dried, finely powdered stem bark (500 g) of *Alseodaphne semicapifolia* was sequentially extracted with petroleum ether, ethyl acetate and ethanol (2×1.5 l, each). The insecticidal petroleum ether extract was concentrated *in vacuo* to obtain a dark brown gummy material (10 g). The purification of crude material was carried out by dry column flash chromatography on silica gel with petroleum ether containing gradiently increasing concentrations of diethyl ether from 0 to 100% (2×25 ml, each). The active fraction afforded the insecticidal compound 5 as a white crystalline solid (70 mg, 0.014 %, m.p. 123.5°C).

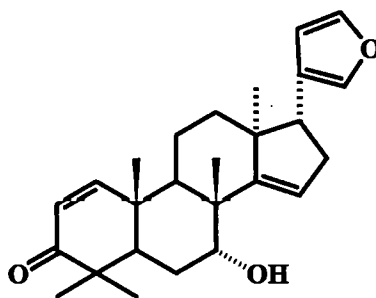
ν_{max} (CHCl_3 , cm^{-1}) ; 2820, 1609, 1480, 1430, 1245, 1080, 780. λ_{max} (EtOH, nm) ; 284, 234.

δ_H (CDCl₃); δ 3.04 (2H, br s, 2 \times CH), 3.85 (2H, dd, J 9.2, 3.6, 2 \times CH₂), 4.22 (2H, dd, J 6.7, 9.2, 2 \times CH₂), 4.70 (2H, d, J 4.2, 2 \times CH), 5.94 (4H, s, 2 \times CH₂), 6.79 (4H, m, 4 \times CH), 6.84 (2H, d, J 0.91, 2 \times CH).

δ_C (CDCl₃); 54.2 (2 \times CH), 71.6 (2 \times CH₂), 85.7 (2 \times CH), 101.0 (2 \times CH₂), 106.4 (2 \times CH), 108.1 (2 \times CH), 119.3 (2 \times CH), 134.9 (2 \times C_q), 147.0 (2 \times C_q), 147.9 (2 \times C_q). Mass Spec. (EI) m/z 354 M⁺.

3.5 EXTRACTION AND ISOLATION OF INSECTICIDAL COMPOUNDS FROM *WALSURA PISCIDIA*

3.5.1 Isolation of compound 6



The shade-dried, finely powdered stem bark (500 g) of *Walsura piscidia* was sequentially extracted with petroleum ether, ethyl acetate and ethanol (2 \times 1.5 l, each). The insecticidal petroleum ether extract was concentrated *in vacuo* to obtain a light yellow gummy material (10 g). The purification of crude material was carried out by dry column flash chromatography on silica gel with petroleum ether containing gradiently increasing concentrations of diethyl ether from 0 to 100% (2 \times 25 ml, each). The active fraction obtained from dry

column flash chromatography afforded the insecticidal compound **6** as a white crystalline solid (70 mg, 0.014%, m.p. 194-195 °C).

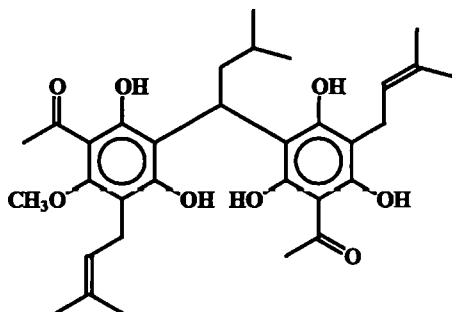
ν_{\max} (CHCl₃, cm⁻¹) ; 3587, 1704, 1665. λ_{\max} (EtOH, nm) ; 216.

δ_{H} (CDCl₃); δ 0.8 (3H, s), 1.10 (3H, s), 1.16 (2H, m), 1.18 (6H, s), 1.60-1.82 (2H, m), 2.22 (2H, m), 2.41 (2H, m), 2.60 (2H, s). 2.86 (1H, dd, *J* 7.3, 7.2, CH), 4.17 (1H, d, *J* 7.3, CH), 5.60 (1H, d, *J* 2.4, CH), 5.84 (1H, d, *J* 10.0, CH), 6.29 (1H, s, CH), 7.14 (1H, d, *J* 10.3, CH), 7.27 (1H, s, CH), 7.38 (1H, t, *J* 1.8, CH).

δ_{C} (CDCl₃); (CDCl₃) δ 16.2 (CH₂), 18.9 (CH₃), 20.2 (CH₃), 21.5 (CH₃), 24.2 (CH₂), 27.1 (CH₃), 27.5 (CH₃), 32.2 (CH₂), 34.3 (CH₂), 36.9 (CH), 40.2 (C_q), 44.2 (CH), 44.4 (C_q), 44.8 (C_q), 47.3 (C_q), 51.5 (CH), 71.5 (CH), 111.0 (CH), 120.0 (CH₂), 124.2 (C_q), 125.4 (CH), 139.7 (CH), 142.6 (CH), 158.2 (CH), 161.2 (C_q), 205.1 (C_q). Mass Spec. (EI) *m/z* 394 M⁺.

3.6 SYNTHESIS PROCEDURES

Synthesis of 1-[(2,6-dihydroxy-4-methoxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl)-1-(2,4,6-trihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)phenyl)]-3-methylbutane, (acrovestone), (1)

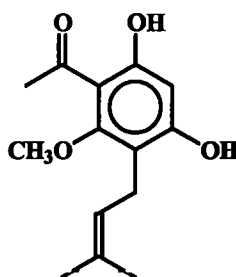


A solution of acronylin (**2.5**) (1.0 g, 4.0 mmol), demethylacronylin (**2.6**) (0.94 g, 4.0 mmol), isovaleraldehyde (0.20 g, 2.3 mmol) and Amberlyst 15 (0.50 g) was heated to reflux in dichloromethane:diethyl ether (4:1, 30 ml) for 30 min. Amberlyst was removed by filtration and the filtrate concentrated *in vacuo*. The crude product was purified by dry column flash chromatography using a mixture of diethyl ether:petroleum ether (3:7, v/v) to afford the title compound **1** (0.40 g, 30%) as a pale yellow crystalline solid, m.p. 142-143 °C.

ν_{\max} (KBr, cm^{-1}); 3393, 3246, 2968, 2954, 1601, 1461, 1363, 1320; δ_{H} (CDCl_3); 0.84 (6H, br s, $2 \times \text{CH}_3$), 1.41 (1H, m, CH), 1.69 (3H, s, CH_3), 1.77 (6H, s, $2 \times \text{CH}_3$), 1.84 (3H, s, CH_3), 2.24 (2H, m, CH_2), 2.67 (3H, s, CH_3), 2.71 (3H, s, CH_3), 3.33 (2H, d, J 6.6, CH_2), 3.40 (2H, d, J 6.6, CH_2), 3.71 (3H, s, CH_3), 4.74 (1H, t, J 7.7, CH), 5.20 (2H, J 6.6, $2 \times \text{CH}$), 6.20 (1H, br s, OH),

9.58 (1H, br s, OH), 10.06 (1H, br s, OH), 15.62, (1H, br s, OH), 15.70 (1H, br s, OH); δ_c (CDCl₃); 17.9 (CH₃), 18.0 (CH₃), 22.2 (CH₃), 22.3 (CH₂), 22.5 (CH₃), 23.2 (CH₂), 25.5 (CH₃), 25.6 (CH₃), 27.2 (CH), 28.8 (CH), 30.6 (CH₃), 32.6 (CH₃), 39.6 (CH₂), 62.6 (CH₃), 104.9 (C_q), 106.3 (C_q), 108.3 (C_q), 108.8 (C_q), 113.2 (C_q), 116.8 (C_q), 121.7 (CH), 123.2 (CH), 131.7 (C_q), 136.7 (C_q), 158.3 (C_q), 158.4 (C_q), 160.3 (C_q), 160.9 (C_q), 204.2 (C_q), 204.3 (C_q). Mass spec. (EI) m/z 554 M^+ .

Synthesis of 1-[4,6-dihydroxy-2-methoxy-3-(3-methyl-but-2-enyl)-phenyl]-ethanone, (acronylin) (2.5)



Method (i)

To a solution of 4,6-dihydroxy-2-methoxyacetophenone (**2.15**) (6.0 g, 3.5 mmol) in absolute dioxane (15 ml), BF₃-Et₂O (2 ml) was added slowly. The reaction mixture was warmed to 40 °C and stirred for 30 min. A solution of 2-methyl-but-3-ene-2-ol (3.0 g, 3.5 mmol) in dry dioxane (3 ml) was added to this mixture and stirred for another 2 h. On cooling, diethyl ether (50 ml) was added and the solution washed with water (3 × 50 ml). The organic layer was dried over MgSO₄ and concentrated *in vacuo*. The resulting gum was purified

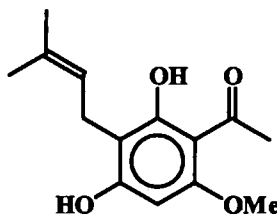
by column chromatography on silica gel using a mixture of diethyl ether:petroleum ether (1:1, v/v) to obtain the desired product (2.5) (800 mg, 12%) as a white crystalline solid, m.p. 126-127 °C and compound 2.19 (0.750 g, 10%) as a white crystalline solid m.p. 169-171 °C.

Method (ii)

Compound (2.26) (0.50 g, 1.6 mmol) in 50% aqueous KOH (5 ml) and DMSO (10 ml) was heated to 115 °C for 2 h. On cooling, 10% HCl (20 ml) was added and the solution was extracted with diethyl ether (3 × 50 ml). The combined extracts were washed with water (3 × 50 ml), dried over MgSO₄ and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel using a mixture of diethyl ether:petroleum ether (1:1, v/v) to afford compound (2.5) (350 mg, 87%) as a white crystalline solid.

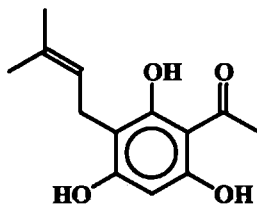
ν_{max} (KBr, cm⁻¹); 3289, 1625, 1590, 1413, 1363, 1233; δ_{H} (CD₃OD); 1.72 (3H, s, CH₃), 1.75 (3H, s, CH₃), 2.63 (3H, s, CH₃), 3.21 (2H, d, *J* 7.0, CH₂), 3.82 (3H, s, OCH₃), 5.23 (1H, t, *J* 7.0, CH), 6.22 (1H, s, Ar-H), 9.63 (1H, br s, OH), 13.31 (1H, br s, OH); δ_{C} (CD₃OD); 17.9 (CH₃), 23.4 (CH₂), 25.6 (CH₃), 31.0 (CH₃), 63.2 (CH₃), 99.9 (CH), 109.5 (C_q), 115.9 (C_q), 124.5 (CH), 131.7 (C_q), 162.9 (C_q), 164.9 (2 × C_q), 204.4 (C_q). Mass spec. (EI) *m/z* 250 M⁺.

1-[2,4-dihydroxy-6-methoxy-3-(3-methyl-but-2-enyl)-phenyl]-ethanone
(preremirol) (2.19)



ν_{\max} (KBr, cm^{-1}); 3290, 1595, 1458, 1122; δ_{H} (CDCl_3); 1.65 (3H, s, CH_3), 1.76 (3H, s, CH_3), 2.60 (3H, s, CH_3), 3.25 (2H, d, J 6.8, CH_2), 3.88 (3H, s, OCH_3), 5.17 (1H, t, J 6.8, CH), 5.90 (1H, s, OH), 13.94 (1H, s, OH); δ_{C} (CDCl_3); 17.7 (CH_3), 21.8 (CH_2), 25.8 (CH_3), 33.1 (CH_3), 55.3 (CH_3), 94.8 (CH), 105.2 (C_q), 109.4 (C_q), 122.6 (CH), 131.4 (C_q), 162.6 (C_q), 162.8 (C_q), 162.7 (C_q), 204.3 (C_q). Mass spec. (EI) m/z 250 M^+ .

Synthesis of 1-[2,4,6-trihydroxy-3-(3-methyl-but-2-enyl)-phenyl]-ethanone
(demethylacronylin) (2.6)



Method (i)

To an ice cold stirred solution of 2,4,6-trihydroxyacetophenone (7.0 g, 50 mmol) in 10% aqueous KOH (degassed over 3 h, 225 ml), was added prenyl

bromide (6.2 g, 50 mmol) over 30 min. The reaction mixture was stirred for an additional 3 h at room temperature under N_2 . After this time the reaction mixture was neutralised with 10% HCl (300 ml). The aqueous layer was extracted with diethyl ether (3×50 ml). The combined extracts were washed with brine, water (3×50 ml, each), dried over $MgSO_4$ and concentrated *in vacuo*. Dichloromethane (30 ml) was added to the gummy crude material and the yellow residue obtained was recrystallised with a mixture of diethyl ether:petroleum ether (9:1, v/v) to obtain the desired product (2.6) (3.5 g, 55%) as a yellow crystalline solid, m.p. 171-172 °C.

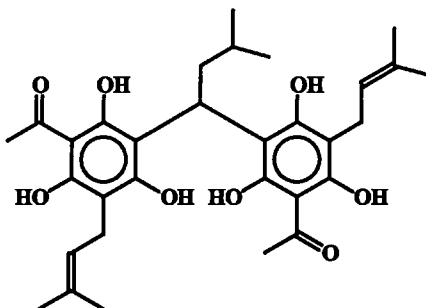
Method (ii)

To a stirred solution of dried 2,4,6 trihydroxyacetophenone (1.0 g, 5 mmol) in dry dioxane (8 ml) was added gradually $BF_3 \cdot Et_2O$ etherate (0.3 ml) at 50 °C. When the solution had acquired a pink red colour, a solution of 2-methyl-but-3-en-2-ol (86 mg, 5 mmol) in dry dioxane (2 ml) was added. The mixture was stirred for 40 min. On cooling, the reaction mixture was diluted with diethyl ether (50 ml) and washed with $NaHCO_3$ solution, brine and water (3×50 ml, each). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude mixture was purified by column chromatography on silica gel eluting with methanol:dichloromethane (1:99, v/v) to obtain the title compound (2.6) (152 mg, 12%) as a yellow crystalline solid.

ν_{max} (KBr, cm^{-1}); 3344, 1634, 1598, 1560, 1448, 1149; δ_H (CD_3COCD_3); 1.62 (3H, s, CH_3), 1.72 (3H, s, CH_3), 2.61 (3H, s, CH_3), 3.23 (2H, d, J 7.2, CH_2),

5.22 (1H, t, J 7.2, CH), 6.07 (1H, s, Ar-H), 9.09 (1H, s, OH), 13.94 (1H, s, OH), 13.96 (1H, s, OH); δ_{C} (CD_3COCD_3); 17.8 (CH_3), 21.8 (CH_2), 25.8 (CH_3), 32.8 (CH_3), 94.8 (CH), 105.2 (C_q), 107.4 (C_q), 124.4 (CH), 130.7 (C_q), 160.6 (C_q), 162.7 (C_q), 164.8 (C_q), 203.4 (C_q). Mass spec. (EI) m/z 236 M^+ .

Synthesis of 1,1-di-[3-oxoethyl-5-(3-methyl-but-2-enyl)-2,4,6-trihydroxyphenyl]-3-methylbutane (demethylacrovestone) (2.10)

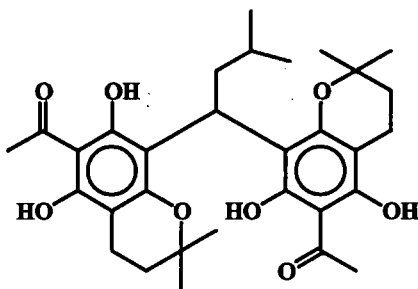


To a solution of demethylacronylin (2.6) (0.5 g, 2.1 mmol) in a mixture of dichloromethane:diethyl ether (4:1, 20 ml) and Amberlyst 15 (0.1 g) was added dropwise isovaleraldehyde (0.92 mg, 1.1 mmol). The mixture was stirred at room temperature for 12 h. Amberlyst was removed by filtration and the filtrate was concentrated *in vacuo*. The crude product was purified by dry column flash chromatography eluting with diethyl ether:petroleum ether (3:7, v/v) to obtain the title compound (2.10) (0.2 g, 45%) as a yellow crystalline solid, m.p 173-174 °C and the compound 2.49 (85 mg, 15%) as a yellow gummy semisolid.

ν_{max} (KBr, cm^{-1}); 3300, 1650, 1620, 1580, 1445, 1360, 1320; δ_{H} (CDCl_3); 0.87 (6H, br s, $2 \times \text{CH}_3$), 1.40 (1H, m, CH), 1.78 (6H, s, $2 \times \text{CH}_3$), 1.84 (6H, s, $2 \times$

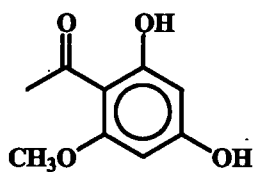
CH₃), 2.23 (2H, m, CH₂), 2.67 (6H, s, 2 × CH₃), 3.41 (4H, d, *J* 7.1, 2 × CH₂), 4.71 (1H, t, *J* 7.8, CH), 5.22 (2H, t, *J* 7.1, 2 × CH); δ_C (CDCl₃); 17.8 (CH₃), 17.9 (CH₃), 22.5 (2 × CH₃), 25.85 (2 × CH₃), 28.3 (CH), 32.7 (2 × CH₃), 39.5 (CH₂), 105.0 (C_q), 105.1 (C_q), 106.1 (C_q), 106.3 (C_q), 109.5 (2 × C_q), 121.4 (CH), 121.7 (CH), 135.5 (C_q), 136.8 (C_q), 158.1 (2 × C_q), 160.0 (C_q), (161.1 (2 × C_q), 161.2 (C_q), 203.7 (2 × C_q). Mass spec. (EI) *m/z* 540 M⁺.

1,1-di-[2,4-dihydroxy-7,7-dimethyl-chroman-3-oxo ethyl]-3-methyl butane (2.47)



ν_{\max} (KBr, cm⁻¹); 3320, 1650, 1610, 1580, 1410, 1320; δ_H (CDCl₃); 0.86 (6H, d, *J* 7.2, 2 × CH₃), 1.33 (6H, s, 2 × CH₃), 1.35 (6H, s, 2 × CH₃), 1.73 (1H, m, CH), 1.80 (2H, t *J* 6.5, CH₂), 2.59 (2H, t *J* 6.8, CH₂), 2.63 (6H, s, 2 × CH₃), 4.70 (1H, t, *J* 7.7, CH), 9.20 (1H, s, OH), 14.9 (1H, br s, OH), 15.00 (1H, br s, OH); δ_C (CDCl₃); 16.7 (CH₂), 16.9 (CH₂), 22.5 (CH₃), 22.6 (CH₃), 26.7 (2 × CH₃), 26.8 (2 × CH₃), 31.7 (2 × CH₃), 33.0 (CH₂), 39.4 (CH), 76.0 (2 × C_q), 102.0 (C_q), 107.9 (C_q), 156.0 (C_q), 160.7 (C_q), 161.3 (C_q), 161.5 (C_q), 203.6 (C_q), 203.9 (C_q).

Synthesis of 1-(4,6-dihydroxy-2-methoxy-phenyl)-ethanone (2.15)

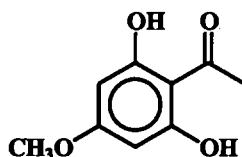


Acetyl Chloride (560 mg, 7 mmol) was added dropwise to a stirred solution of 5-methoxyresorcinol (**2.14**) (1.0 g, 7 mmol) and AlCl_3 (2.7 g, 21 mmol) in dry nitrobenzene at 0°C under N_2 . After 10 min, the solution was heated to reflux for 2 h. On cooling, the mixture was poured onto ice cold water (25 ml) and extracted with diethyl ether (3×50 ml). The combined organic extracts were extracted with 10% aqueous NaOH solution (3×50 ml). The aqueous extracts were neutralised with 2N HCl and extracted with diethyl ether (3×50 ml). The combined organic extracts were washed with water (3×50 ml), dried over MgSO_4 and evaporated *in vacuo*. The crude product was purified by dry column flash chromatography using a mixture of diethyl ether:petroleum ether (1:1, v/v) to afford the title compound **2.15** (549 mg, 42.8%) as a white crystalline solid, m.p. $173\text{--}174^\circ\text{C}$ and compounds **2.17** (274 mg, 21%) and **2.18** (176 mg, 11.2%) as white crystalline solids, m.p. $139\text{--}141^\circ\text{C}$, $102\text{--}104^\circ\text{C}$, respectively.

ν_{max} (KBr, cm^{-1}); 3549, 3149, 1635, 1558, 418; δ_{H} (CD_3COCD_3); 2.54 (3H, s, CH_3), 3.90 (3H, s, OCH_3), 5.95 (1H, d, J 2.2, Ar-H), 6.02 (1H, d, J 2.2, Ar-H), 9.51 (1H, br s, OH), 13.95 (1H, br s, OH); δ_{C} (CD_3COCD_3) 32.9 (CH_3), 56.0

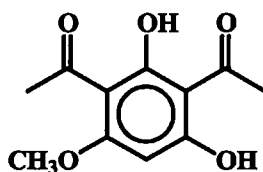
(CH₃), 91.7 (CH), 96.6 (CH), 105.9 (C_q), 164.7 (C_q), 165.7 (C_q), 168.2 (C_q), 203.4 (C_q). Mass spec. (EI) m/z 182 M⁺.

1-(2,6-dihydroxy-4-methoxy-phenyl)-ethanone (2.17)



ν_{\max} (KBr, cm⁻¹); 3133, 1652, 1558, 1258, 418; δ_{H} (CD₃COCD₃); 2.59 (3H, s, CH₃), 4.01 (3H, s, OCH₃) 5.99 (2H, s, 2 × Ar-H), 14.77 (1H, s, OH); δ_{C} (CD₃COCD₃); 33.0 (CH₃), 56.8 (CH₃), 92.1 (CH), 104.7 (C_q), 105.0 (C_q), 168.4 (C_q), 172.4 (C_q), 172.9 (C_q), 204.4 (C_q), 204.8 (C_q). Mass spec. (EI) m/z 182 M⁺

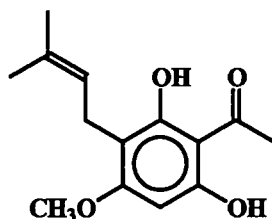
1-(2,6-dihydroxy-4-methoxy-3-oxoethyl-phenyl)-ethanone (2.18)



ν_{\max} (KBr, cm⁻¹); 3446, 3099, 2935, 1558.

δ_{H} (CD₃COCD₃); 2.59 (3H, s, CH₃), 2.63 (3H, s, CH₃), 4.01 (3H, OCH₃), 5.99 (1H, s, Ar-H), 14.77 (1H, s, OH); δ_{C} (CD₃COCD₃); 32.8 (CH₃), 33.0 (CH₃), 56.0 (CH₃), 91.3 (CH), 104.2 (C_q), 105.0 (C_q), 167.1 (C_q), 171.6 (C_q), 172.0 (C_q), 203.3 (C_q), 204.2 (C_q). Mass spec. (EI) m/z 224 M⁺.

Synthesis of 1-[(2,6-dihydroxy-4-methoxy-3-(3-methyl-2-butenyl)-phenyl]-ethanone (2.20)

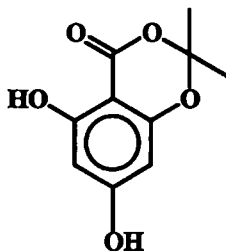


To a solution of 2,6-dihydroxy-4-methoxyacetophenone (2.17) (1.0 g, 5 mmol) in absolute dioxane (15 ml), $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (2 ml) in absolute dioxane (4 ml) was added slowly. The reaction mixture was warmed to 40 °C and stirred for 30 min. A solution of 2-methyl-3-butene-2-ol (0.50 g, 5 mmol) in dry dioxane (4 ml) was added and the mixture stirred for another 2 h. The reaction mixture was cooled to room temperature, diluted with diethyl ether (50 ml), and washed with water (3 × 50 ml). The organic layer was dried over MgSO_4 and concentrated *in vacuo*. The resulting gum was purified by column chromatography on silica gel using diethyl ether:petroleum ether (1:9, v/v) to obtain the desired product (2.20) (80 mg, 12%) as a white crystalline solid. m.p. 124-126 °C.

ν_{max} (KBr, cm^{-1}); 3289, 1625, 1590, 1413, 1363, 1233; δ_{H} (CD_3OD); 1.71 (3H, s, CH_3), 1.76 (3H, s, CH_3), 2.65 (3H, s, CH_3), 3.26 (2H, d, J 7.0, CH_2), 3.85 (3H, s, CH_3), 5.22 (1H, t, J 7.0, CH), 6.20 (1H, s, Ar-H), 9.53 (1H, s, OH), 13.61 (1H, s, OH); δ_{C} (CD_3OD); 17.8 (CH_3), 23.4 (CH_2), 25.6 (CH_3),

31.0 (CH₃), 63.2 (CH₃), 99.9 (CH), 109.5 (C_q), 115.9 (C_q), 124.5 (CH), 131.7 (C_q), 162.9 (C_q), 164.9 (2×C_q), 204.3 (C_q). Mass spec. (EI) m/z 250 M⁺.

Synthesis of 5,7-dihydroxy-2,2-dimethyl 4*H*-1,3-benzodioxin-4-one (2.22)

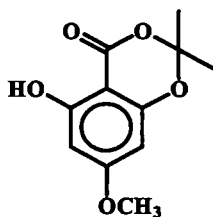


To an ice cold suspension of 2,4,6-trihydroxybenzoic acid monohydrate (2.21) (8.0 g, 2.7 mmol) in TFA (40 ml) were added TFAA (25 ml) and dry acetone (5 ml). The mixture was warmed slowly to room temperature and stirred for 4 h. The resulted slightly yellow solution was concentrated, poured into a saturated solution of NaHCO₃ and extracted with ethyl acetate (3 × 100 ml). The combined organic extracts were washed with water, brine, then dried with MgSO₄ and concentrated *in vacuo* to yield a yellow solid. The crude product was purified by column chromatography on silica gel with diethyl ether:petroleum ether (1:1, v/v) to afford the desired compound (2.22) (9.0 g, 95%) as a white crystalline solid, m.p. 205-207 °C.

ν_{\max} (KBr, cm⁻¹); 3583, 3023, 1687, 1643, 1597; δ_{H} (CD₃COCD₃); 1.72 (6H, s, 2 × CH₃), 6.08 (1H, d, *J* 2.4, Ar-H), 6.09 (1H, d, *J* 2.2, Ar-H), 9.74 (1H, br s,

OH), 10.46 (1H, s, OH); δ_C (CD₃COCD₃); 25.6 (2 × CH₃), 93.0 (C_q), 97.9 (CH), 98.0 (CH), 107.6 (C_q), 158.1 (C_q), 164.0 (C_q), 165.8 (C_q), 167.2 (C_q).

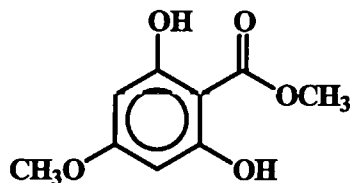
Synthesis of 2,2-dimethyl-5-hydroxy-7-methoxy-4*H*-1,3-benzodioxin-4-one (2.23)



To an ice cold solution of diol (2.22) (3.5 g, 16.6 mmol) and methanol (0.6 g, 17 mmol) in dry THF (50 ml) were added PPh₃ (4.5 g, 17 mmol) and DEAD (3.5 g, 17 mmol) and the mixture was warmed to room temperature over 2 h. The solution was diluted with diethyl ether (50 ml), washed with water (50 ml), dried over MgSO₄ and concentrated *in vacuo*. The crude product was purified by dry column flash chromatography over silica gel using a mixture of petroleum ether:diethyl ether (1:1, v/v) to afford the desired compound (2.23) (2.0 g, 54%) as a white crystalline solid, m.p. 102-103 °C.

ν_{\max} (KBr, cm⁻¹); 3200, 3027, 2999, 2943, 1685, 1638, 1583, 1512, 1358; δ_H (CDCl₃); 1.73 (6H, s, 2 × CH₃), 3.81 (3H, s, OCH₃), 6.00 (1H, d, *J* 2.4, Ar-H), 6.14 (1H, d, *J* 2.1, Ar-H), 10.44 (1H, s, OH); δ_C (CDCl₃); 25.6 (2 × CH₃), 55.7 (CH₃), 93.1 (C_q), 95.6 (CH), 95.7 (CH), 106.9 (C_q), 156.9 (C_q), 163.0 (C_q), 165.2 (C_q), 167.7 (C_q). Mass spec. (EI) *m/z* 224 M⁺.

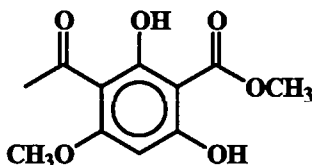
Synthesis of methyl 2,6-dihydroxy-3-methoxybenzoate (2.24)



To a solution of 1,3-benzodioxin-4-one (2.23) (1.8 g, 8 mmol) in dry THF (50 ml) was added NaOMe (2.5 g, 46 mmol) and the mixture was heated to reflux for 4 h. On cooling, 10% HCl (50 ml) was added. The aqueous layer was extracted with diethyl ether (3 × 50 ml), and the combined extracts washed with water (3 × 50 ml), brine, then dried over MgSO₄ and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel eluting with diethyl ether:petroleum ether (1:1, v/v) to afford the compound (2.24) (1.2 g, 80%) as a white crystalline solid, m.p. 103-104 °C.

ν_{\max} (KBr, cm⁻¹); 3440, 3030, 2956, 2849, 1677, 1641, 1582, 1358, 1307; δ_{H} (CDCl₃); 3.82 (3H, s, OCH₃), 4.04 (3H, s, COOCH₃), 6.04 (2H, s, 2 × Ar-H), 10.01 (2H, br s, 2 × OH); δ_{C} (CDCl₃); 52.5 (CH₃), 55.5 (CH₃), 93.9 (C_q), 94.5 (2 × CH), 162.2 (2 × C_q), 166.5 (C_q), 169.8 (C_q). Mass spec. (EI) m/z 198 M⁺.

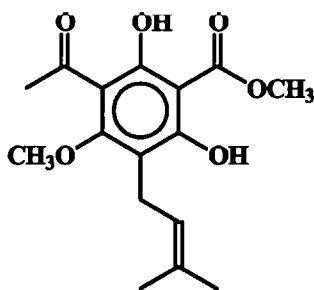
Synthesis of methyl 3-oxoethyl-4-methoxy-2,6-dihydroxybenzoate (2.25)



Titanium tetrachloride (1.0 g, 5.2 mmol) and AcCl (0.4 g, 5.2 mmol) were stirred at 0°C under N_2 and methyl methoxybenzoate (**2.24**) (1.0 g, 5.2 mmol) in benzene (10 ml) was added dropwise. The mixture was stirred at room temperature for another 20 min and 2N HCl (20 ml) was added. The organic layer was diluted with diethyl ether (25 ml) and washed with saturated NaHCO_3 , brine, water, then dried over MgSO_4 and concentrated *in vacuo* to obtain the gummy residue. The crude products was purified by column chromatography on silica using a mixture of diethyl ether:petroleum ether (3:7) to give the desired product (**2.25**) (1.1 g, 91%) as a white crystalline solid, m.p. $140\text{--}142^\circ\text{C}$.

ν_{max} (KBr, cm^{-1}); 3446, 3023, 2979, 2873, 1653, 1641, 1582, 1307, 1358; δ_{H} (CDCl_3); 2.61 (3H, s, CH_3), 3.81 (3H, s, OCH_3), 4.00 (3H, s, COOCH_3), 6.04 (1H, s, Ar-H), 12.52 (1H, br s, OH), 14.21 (1H, br s, OH); δ_{C} (CDCl_3); 33.0 (CH_3), 52.5 (CH_3), 55.9 (CH_3), 91.2 (C_q), 95.5 (C_q), 166.5 (C_q), 169.8 (C_q), 171.6 (C_q), 183.5 (C_q), 210.3 (C_q). Mass spec. (EI) m/z 240 M^+ .

Synthesis of Methyl 3-oxoethyl-1-4-methoxy-5-(3-methyl-2-butenyl)-2,6-dihydroxybenzoate (2.26)



Method (i)

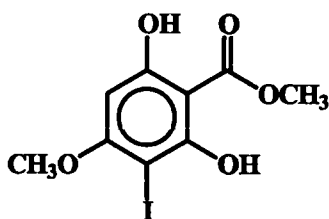
To a solution of methoxybenzoate (**2.25**) (1.0 g, 4.1 mmol) in freshly prepared and degassed 10% aqueous KOH (20 ml) prenyl bromide (1.0 g, 5 mmol) was added dropwise over 30 min. The reaction mixture was stirred for another 2 h, under N₂, at 0 °C. The reaction mixture was acidified with 10% HCl, extracted with diethyl ether (3 × 50 ml) and the combined extracts were washed with NaHCO₃, water, then dried over MgSO₄ and concentrated *in vacuo*. The crude product was purified by column chromatography on silica using a mixture of diethyl ether: petroleum ether (1:1) to afford the title compound (**2.26**) (384 mg, 30 %) as a white crystalline solid, m.p. 135-136 °C.

Method (ii)

A solution of compound (2.29) (500 mg, 1.5 mmol) in ethanol (20 ml) was stirred under H_2 in the presence of Pd/C (10%) for 3 h at room temperature. The solution was filtered and filtrate concentrated *in vacuo*. The crude product was dissolved in toluene (15 ml) and heated to reflux with pTSA (100 mg) for 2 h. On cooling, diethyl ether was added (20 ml), washed with water and brine, then dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by dry column chromatography over silica gel eluting with diethyl ether:petroleum ether (1:1, v/v) to afford the title compound (2.26) (406 mg, 86 %) as a white crystalline solid.

ν_{max} (KBr, cm^{-1}); 3445, 3023, 2959, 2819, 1452; δ_H ($CDCl_3$); 1.72 (3H, s, CH_3), 1.75 (3H, s, CH_3), 2.62 (3H, s, CH_3), 3.21 (2H, d, J 7.0, CH_2), 3.82 (3H, s, CH_3), 4.04 (3H, s, CH_3), 5.23 (1H, t, J 7.1, CH), 12.63 (1H, s, OH), 13.21 (1H, s, OH); δ_C ($CDCl_3$); 17.9 (CH_3), 23.4 (CH_2), 25.9 (CH_3), 31.6 (CH_3), 55.4 (CH_3), 63.2 (CH_3), 108.6 (C_q), 109.4 (C_q), 115.8 (C_q), 124.6 (CH), 131.7 (C_q), 162.9 (C_q), 164.5 (C_q), 164.8 (C_q), 169.4 (C_q), 204.5 (C_q).

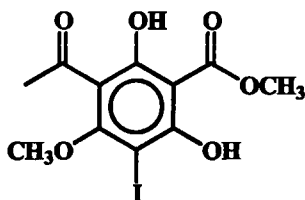
Synthesis of methyl 2,6-dihydroxy-4-methoxy-5-iodobenzoate (2.27)



A solution of **2.24** (1.0 g, 5 mmol) and NIS (1.1 g, 5 mmol) in dry acetonitrile (25 ml) was stirred at room temperature for 2 h. The solvent was evaporated and the crude product was purified by dry column flash chromatography on silica gel using a mixture of diethyl ether:petroleum ether (2:3, v/v) to obtain the compound (**2.27**) (1.5 g, 93%) as a white crystalline solid, m.p. 140-141 °C.

ν_{\max} (KBr, cm^{-1}); 3540, 3023, 1650, 1582; δ_{H} (CDCl_3); 3.82 (3H, s, OCH_3), 4.04 (3H, s, COOCH_3), 5.91 (H, s, Ar-H), 10.01 (2H, br s, $2 \times \text{OH}$); δ_{C} (CDCl_3); 52.5 (CH_3), 55.5 (CH_3), 86.1 (C_q), 93.9 (C_q), 94.5 (CH), 162.2 ($2 \times \text{C}_q$), 166.5 (C_q), 169.8 (C_q).

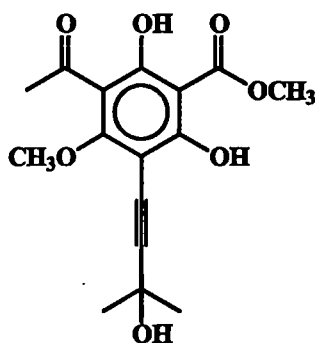
Synthesis of methyl 2,6-hydroxy-4-methoxy-5-3-oxoethyl-iodobenzoate
(2.28)



To an stirred solution of TiCl_4 (0.6 ml, 3.1 mmol) and AcCl (240 mg, 3.1 mmol) at 0°C under N_2 , methyl methoxyiodobenzoate (2.27) (1.0 g, 3.0 mmol) in benzene (10 ml) was added dropwise. The mixture was stirred at room temperature for 2 h and 2N HCl (20 ml) was added. The organic layer was diluted with diethyl ether (25 ml) and washed with saturated NaHCO_3 , brine, water, then dried over MgSO_4 and concentrated in *vacuo* to obtain an off white solid. The solid was purified by column chromatography on silica gel eluting with a mixture of diethyl ether in petroleum ether (2:3, v/v) to afford the title compound (2.28) (1.0 g, 90%) as a white crystalline solid, m.p. $145\text{--}146^\circ\text{C}$.

ν_{max} (KBr, cm^{-1}); 3393, 3246, 2954, 1611, 1461; δ_{H} (CDCl_3); 3.82 (3H, s, OCH_3), 4.04 (3H, s, CH_3), 10.01 (2H, br s, $2 \times \text{OH}$). δ_{C} (CDCl_3); 31.2 (CH_3), 52.5 (CH_3), 55.5 (CH_3), 93.9 (C_q), 94.5 (C_q), 86.1 (C_q), 166.5 (C_q), 169.8 (C_q).
Mass spec. (EI) m/z 366 M^+ .

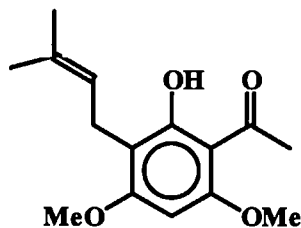
Synthesis of methyl 2,6-dihydroxy-3-oxoethyl-4-methoxy-5-(3-hydroxy-3-methylbutynyl)benzoate (2.29)



To a solution of methyl iodobenzoate (**2.28**) (800 mg, 2.1 mmol) in dry Et₃N (5 ml) was added CuI (16 mg, 4.0% mol), dichloro-*bis*-triphenylphosphene palladium(0) (15 mg, 1% mol) and 2-methyl-but-3-yne-2-ol (183 mg, 2.1 mmol). After refluxing for 14 h the mixture was cooled and concentrated *in vacuo* to obtain a residue which was separated by chromatography using a mixture of diethyl ether:petroleum ether (1:1, v/v) as an eluent to yield the title compound (**2.29**) (600 mg, 85%) as a white crystalline solid, m.p. 134-135 °C.

ν_{\max} (KBr, cm⁻¹); 3448, 3025, 1655, 1600, 1350; δ_{H} (CDCl₃); 1.63 (6H, s, 2 × CH₃), 2.61 (3H, s, CH₃), 2.85 (1H, s, OH), 3.62 (3H, s, CH₃), 4.06 (3H, s, CH₃), 14.61 (1H, s, OH); δ_{C} (CDCl₃); 31.6 (2 × CH₃), 33.2 (CH₃), 53.0 (CH₃), 65.8 (C_q), 66.2 (CH₃), 74.3 (C_q), 93.5 (C_q), 93.9 (C_q), 101.0 (C_q), 104.2 (C_q), 163.2 (C_q), 164.6 (C_q), 164.8 (C_q), 169.5 (C_q), 203.2 (C_q).

Synthesis of 1-[4-6-dimethoxy-2-hydroxy-3-(3-methyl-2-butenyl)-phenyl]-ethanone (2.30)



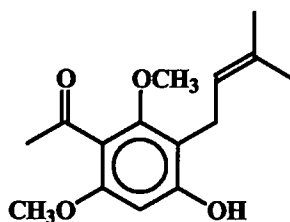
To a solution of 4,6-dimethoxy-2-hydroxyacetophenone (2.33) (2.0 g, 10.1 mmol) in absolute dioxane (15 ml), $\text{BF}_3\text{-Et}_2\text{O}$ (1 ml) was added slowly. The reaction mixture was warmed to 40°C and stirred for 30 min. A solution of 2-methyl-but-3-ene-2-ol (1.0 g, 11.6 mmol) in dry dioxane (2 ml) was added and the mixture stirred for 2 h. The reaction mixture was cooled to room temperature, diluted diethyl ether (50 ml) and washed with water (3×50 ml). The organic layer was dried over MgSO_4 and concentrated *in vacuo*. The resulting gum was purified by column chromatography on silica gel eluting with diethyl ether:petroleum ether (3:7, v/v) to obtain the desired compound (2.30) (320 mg, 12%) as a white crystalline solid, m.p. $112\text{-}113^\circ\text{C}$.

ν_{max} (KBr, cm^{-1}); 3420, 3340, 2980, 2920, 1450.

δ_{H} (CDCl_3); 1.76 (3H, s, CH_3), 1.78 (3H, s, CH_3), 2.61 (3H, s, CH_3), 3.25 (2H, d, J 7.0, CH_2), 3.87 (6H, s, $2 \times \text{CH}_3$), 5.17 (1H, t, J 7.0, CH), 5.92 (1H, s, Ar-H), 13.31 (1H, s, OH); δ_{C} (CDCl_3); 17.7 (CH_3), 21.8 (CH), 25.8 (CH_3), 33.1 (CH_2), 55.3 (CH_3), 55.4 (CH_3), 85.7 (CH), 105.8 (C_q), 109.5 (C_q), 122.7

(CH), 131.3 (C_q), 161.7 (C_q), 163.2 (C_q), 163.4 (C_q), 203.3 (C_q). Mass spec. (EI) m/z 264 M⁺.

Synthesis of 1-[2-6-dimethoxy-4-hydroxy-3-(3-methyl-2-butenyl)-phenyl]-ethanone (2.31)

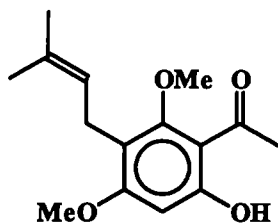


To a solution of 2,6-dimethoxy-4-hydroxyacetophenone (2.37) (1.0 g, 5 mmol) in absolute dioxane (10 ml), BF₃-Et₂O (1 ml,) was added slowly. The reaction mixture was warmed to 40 °C and stirred for 30 min. A solution of 2-methylbut-3-ene-2-ol (0.50 g, 5.8 mmol) in dry dioxane (4 ml) was added and the mixture stirred for another 40 min. The reaction mixture was cooled to room temperature, diluted with diethyl ether (50 ml), and washed with water (3 × 50 ml). The organic layer was dried over MgSO₄ and concentrated *in vacuo*. The resulting gum was purified by column chromatography on silica gel using a mixture of diethyl ether:petroleum ether (3:7, v/v) to obtain the desired compound (2.31) (150 mg, 12%) as a white crystalline solid, m.p. 69-70 °C.

ν_{\max} (KBr, cm⁻¹); 3460, 3000, 1595, 1458; δ_{H} (CDCl₃); 1.76 (3H, s, CH₃), 1.73 (3H, s, CH₃), 2.60 (3H, s, CH₃), 3.25 (2H, d, *J* 7.0, CH₂), 3.85 (6H, s, 2 × OCH₃), 5.22 (1H, t, *J* 7.0, CH), 5.93 (1H, s, Ar-H), 13.21 (1H, s, OH); δ_{C} (CDCl₃); 17.7 (CH₃), 21.7 (CH₂), 25.8 (CH₃), 33.4 (CH₃), 55.4 (OCH₃), 55.5

(OCH₃), 85.9 (CH), 105.6 (C_q), 109.9 (C_q), 122.2 (CH), 131.7 (C_q), 161.7 (C_q), 163.1 (C_q), 163.2 (C_q), 203.4 (C_q).

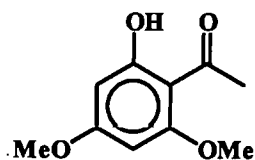
Synthesis of 1-[2,4-dimethoxy-6-hydroxy-(3-methyl-2-butenyl)-phenyl]-ethanone (2.32)



The compound (2.39) (500 mg, 1.8 mmol) was heated at 150-175 °C without solvent under N₂ for 2 h. The crude product was purified by dry column flash chromatography over silica gel using diethyl ether:petroleum ether (3:7, v/v) as eluent to give the title compound (2.32) (400 mg, 80%) as a white crystalline solid, m.p. 69-70 °C.

ν_{\max} (KBr, cm⁻¹); 3334, 1634, 1598, 1282, 1150; δ_{H} (CDCl₃); 1.62 (3H, s, CH₃), 1.72 (3H, s, CH₃), 2.61 (3H, s, CH₃), 3.23 (2H, d, *J* 7.2, CH₂), 5.22 (H, t, *J* 7.2, CH), 6.07 (1H, s, Ar-H), 13.94 (1H, s, OH); δ_{C} (CDCl₃); 17.8 (CH₃), 21.8 (CH₂), 25.8 (CH₃), 32.8 (CH₃), 56.3 (CH₃), 56.8 (CH₃), 94.8 (CH), 105.2 (C_q), 107.4 (C_q), 121.3 (CH), 131.3 (C_q), 160.6 (C_q), 160.8 (C_q), 162.7 (C_q), 203.4 (C_q).

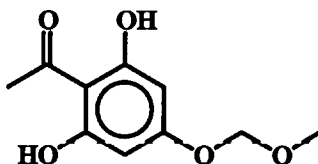
Synthesis of 1-(2,4-dimethoxy-6-hydroxy)-phenylethanone (2.33)



2,4,6-trihydroxyacetophenone (2 g, 11 mmol), dimethyl sulfate (5.0 g) and freshly dried K_2CO_3 (10.0 g) were heated to reflux in dry acetone for 15 h. The K_2CO_3 was removed by filtration and the filtrate was concentrated *in vacuo*. The crude product was purified by column chromatography over silica gel using diethyl ether:petroleum ether (1:1, v/v) to obtain the desired compound (2.33) (2.3 g, 97%) as a white crystalline solid, m.p. 74-75 °C.

ν_{\max} (KBr, cm^{-1}); 3428, 1654, 1558, 1258; δ_H ($CDCl_3$); 2.57 (3H, s, CH_3), 3.79 (3H, s, CH_3), 3.82 (3H, s, CH_3), 5.87 (1H, d, J 2.2, Ar-H), 6.01 (1H, d, J 2.2, Ar-H), 14.03 (1H, br s, OH); δ_C ($CDCl_3$); 32.9 (CH_3), 55.4 (CH_3), 55.5 (CH_3), 90.8 (CH), 93.4 (CH), 105.8 (C_q), 162.9 (C_q), 166.1 (C_q), 167.5 (C_q).

Synthesis of 1-[2,6-dihydroxy-4-methoxymethoxy-phenyl]-ethanone (2.35)

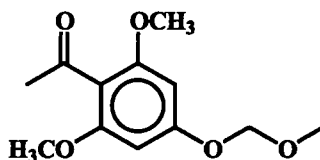


A solution of 2,4,6-trihydroxyacetophenone (2.0 g, 10 mmol), MOMCl (1.0 g, 10 mmol), and K_2CO_3 (2.5 g) in dry acetone (25 ml), was heated to reflux for 2 h. K_2CO_3 was removed by filtration and filtrate was concentrated *in vacuo*.

The crude product was purified by dry column flash chromatography using diethyl ether:petroleum ether (1:4, v/v) to obtain the desired compound (2.35) (1.0 g, 45%) as a white crystalline solid, m.p. 72-73 °C.

ν_{\max} (KBr, cm^{-1}); 3422, 2930, 1650, 1400; δ_{H} (CDCl_3); 2.62 (3H, s, CH_3), 3.86 (3H, s, CH_3), 5.16 (2H, s, CH_2), 6.04 (2H, s, $2 \times \text{Ar-H}$), 9.3 (1H, br s, OH); δ_{C} (CDCl_3); 32.8 (CH_3), 55.0 (CH_3), 90.5 ($2 \times \text{CH}$), 93.9 (CH_2), 105.8 (C_q), 162.8 (C_q), 166.5 ($2 \times \text{C}_q$).

Synthesis of 1-[2,6-dimethoxy-4-methoxymethoxy]-phenyl]-ethanone (2.36)

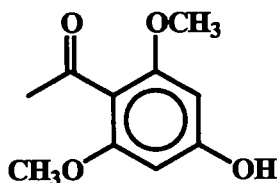


A solution of methoxy methyl ether (2.35) (2.0 g, 9.4 mmol), dimethyl sulphate (4.0 g) and freshly dried K_2CO_3 (5 g) in dry acetone (50 ml) was heated to reflux for 3 h. K_2CO_3 was filtered and the filtrate was concentrated *in vacuo* to obtain the compound 2.36 (2.0 g, 90%) as an oil.

ν_{\max} (CHCl_3 , cm^{-1}); 1650, 1420, 1200; δ_{H} (CDCl_3); 2.62 (3H, s, CH_3), 3.47 (3H, s, CH_3), 3.52 (3H, s, CH_3), 3.86 (3H, s, CH_3), 5.16 (2H, s, CH_2), 6.04 (2H, s, $2 \times \text{Ar-H}$), 9.3 (1H, br s, OH); δ_{C} (CDCl_3); 32.8 (CH_3), 55.0 (CH_3), 55.6

(CH₃), 56.4 (CH₃), 90.5 (2 × CH), 94.2 (CH₂), 105.8 (C_q), 162.8 (C_q), 166.5 (2 × C_q).

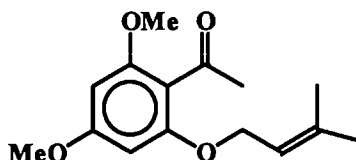
Synthesis of 1-[2,6-dimethoxy-4-hydroxy]-phenyl]-ethanone (2.37)



To a cold solution of compound **2.36** (1.0 g, 2.1 mmol) in methanol (15 ml) was added 10 % aqueous HCl (5 ml) and the mixture was heated to reflux for 30 min. On cooling, the reaction mixture was poured into cold water (20 ml) and extracted with diethyl ether (3 × 20 ml). The combined extracts were washed with water (3 × 20 ml), saturated aqueous NaHCO₃ solution (3 × 20 ml) and dried over MgSO₄ and concentrated *in vacuo*. The crude product was purified by column chromatography on silica using diethyl ether:petroleum ether (1:1, v/v) to afford the title compound (**2.37**) (750 mg, 92%) as an off white crystalline solid, m.p. 70-72 °C.

ν_{max} (KBr, cm⁻¹); 3440, 2995, 1620, 1095; δ_{H} (CDCl₃); 2.59 (3H, s, CH₃), 3.84 (6H, s, 2 × CH₃), 6.03 (2H, s, Ar-H), 9.3 (1H, br s, OH); δ_{C} (CDCl₃); 32.8 (CH₃), 55.4 (2 × CH₃), 90.5 (2 × CH), 105.8 (C_q), 162.8 (C_q), 166.5 (2 × C_q).

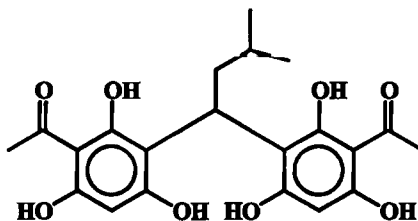
Synthesis of 1-[4,6-dimethoxy-2-(3-methyl-2-butenyloxy)-phenyl]-ethanone (2.38)



A solution of 4,6-dimethoxy-2-hydroxyacetophenone (2.33) (2.0 g, 10 mmol), prenyl bromide (1.5 g, 10 mmol), freshly dried K_2CO_3 (2.0 g) were heated to reflux in dry acetone (30 ml) for 15 h. K_2CO_3 was removed by filtration and filtrate was concentrated *in vacuo*. The crude product was purified by dry column flash chromatography using diethyl ether:petroleum ether (1:1, v/v) to obtain the desired compound (2.38) (1.3 g, 95%) as a yellow oil.

ν_{\max} ($CHCl_3$, cm^{-1}); 1650, 1458, 1230; δ_H ($CDCl_3$); 1.70 (3H, s, CH_3), 1.75 (3H, s, CH_3), 2.45 (3H, s, CH_3), 3.83 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.51 (2H, d, J 6.1, CH_2), 5.41 (1H, t, J 6.1, CH), 6.09 (1H, s, Ar-H), 6.10 (1H, s, Ar-H); δ_C ($CDCl_3$); 18.0 (CH_3), 25.5 (CH_3), 32.3 (CH_2), 55.2 (CH_3), 55.6 (CH_3), 65.5 (CH_3), 90.5 (CH), 91.6 (CH), 113.9 (C_q), 119.3 (CH), 137.5 (C_q), 157.5 (C_q), 158.0 (C_q), 162.0 (C_q), 201.5 (C_q).

Synthesis of 1,1-di-[(2,4,6-trihydroxy-1-oxoethyl)-phenyl]-3-methyl butane (2.40)



Method (i)

A solution of 2,4,6-trihydroxyacetophenone (5.0 g, 26 mmol), isovaleraldehyde (1.0 g, 13 mmol) and Amberlyst 15 (3.0 g) in dichloromethane:diethyl ether (4:1, 100 ml) was heated to gentle reflux for 30 min. Amberlyst residue was removed by filtration and the filtrate was concentrated *in vacuo*. The crude product was purified by column chromatography over silica gel eluting with a mixture of dichloromethane:methanol (1:99, v/v). The desired product (2.40) (3.7 g, 65%) was obtained as a yellow solid and recrystallised with dichloromethane, m.p. 153-154 °C.

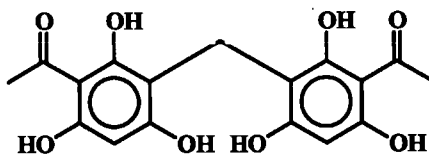
Method (ii)

To a solution of 2,4,6-trihydroxyacetophenone (0.5 g, 2.5 mmol) in DMF (10 ml) was added isovaleraldehyde (37 mg, 1.5 mmol) and trimethyl amine (1.0 g, 10 mmol). The reaction mixture was warmed to 40 °C and stirred for 4 h. On cooling, diethyl ether (20 ml) was added and organic layer was washed with

2M H₂SO₄ (20 ml), water (3 × 20 ml), then dried over Na₂SO₄ and concentrated *in vacuo* to obtain a yellow solid. The solid was purified by column chromatography on silica gel eluting with a mixture of methanol:dichloromethane (1:99, v/v) gave the title compound (**2.40**) (300 mg, 70%).

ν_{\max} (KBr, cm⁻¹); 3420, 3335, 1634, 1598, 1150; δ_{H} (CD₃COCD₃); 0.89 (3H, s, CH₃), 0.91 (3H, s, CH₃), 1.45 (1H, m, CH), 2.10 (2H, m, CH₂), 2.61 (3H, s, CH₃), 4.94 (1H, br s, CH), 5.95 (2H, s, 2 × CH); δ_{C} (CD₃COCD₃); 22.7 (CH₃), 22.8 (CH₃), 27.3 (CH), 28.1 (CH), 32.5 (CH₃), 40.8 (CH₂), 95.6 (2 × CH), 105.3 (2 × C_q), 109.3 (2 × C_q), 162.0 (2 × C_q), 163.9 (2 × C_q).

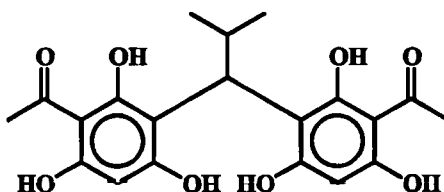
Synthesis of *bis*-2,4,6-trihydroxyacetophenone (**2.41**)



To a solution of 2,4,6-trihydroxyacetophenone (500 mg, 2 mmol) in dichloromethane:diethyl ether (9:1, 25 ml) was added formaldehyde (45 mg, 1 mmol) and Amberlyst 15 (0.5 g) and the mixture heated to reflux for 45 min. On cooling, Amberlyst was filtered and the filtrate was concentrated *in vacuo*. The crude product was purified by column chromatography over C-18 silica gel eluting with a mixture of water:methanol (3:7, v/v) to obtain the desired compound (**2.41**) (250 mg, 60%) as a yellow solid, m.p. 140-141 °C.

ν_{\max} (KBr, cm^{-1}); 3430, 3340, 1282, 1072; δ_{H} (CD_3COCD_3); 2.61 (6H, s, $2 \times \text{CH}_3$), 4.94 (2H, br s, CH), 5.95 (2H, s, $2 \times \text{CH}$); δ_{C} (CD_3COCD_3); 40.8 (CH_2), 95.6 ($2 \times \text{CH}$), 105.3 ($2 \times \text{C}_q$), 109.3 ($2 \times \text{C}_q$), 162.0 ($2 \times \text{C}_q$), 163.9 ($2 \times \text{C}_q$).

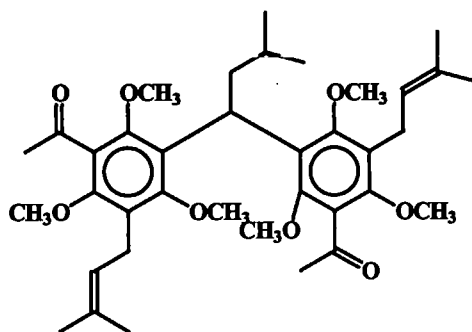
Synthesis of 1,1-di[(1-oxyethyl-2,4,6-trihydroxy)-phenyl]-2-methylpropane (2.43)



To a solution of 2,4,6-trihydroxyacetophenone (0.50 g, 2.0 mmol) in dichloromethane:diethyl ether (4:1, 25 ml) was added isobutraldehyde (107 mg, 1 mmol) and Amberlyst 15 (0.50 g). The mixture was heated to reflux for 90 min. On cooling, Amberlyst residue was filtered and the filtrate was concentrated *in vacuo*. The resulting yellow solid was purified by column chromatography on silica gel using a mixture of dichloromethane:methanol (99:1, v/v) to obtain the desired compound (2.43) (350 mg, 78%) as a yellow solid, m.p. 138-139 °C.

ν_{\max} (KBr, cm^{-1}); 3500, 3435, 1634, 1150; δ_{H} (CD_3COCD_3); 0.85 (6H, d, J 6.3, $2 \times \text{CH}_3$), 2.65 (6H, s, $2 \times \text{CH}_3$), 3.10 (1H, m, CH), 4.27 (1H, d, J 11.2, CH), 6.04 (2H, s, $2 \times \text{Ar-H}$); δ_{C} (CD_3COCD_3); 22.3 ($2 \times \text{CH}_3$), 28.2 (CH), 32.6 (CH_3), 39.2 (CH), 97.0 ($2 \times \text{CH}$), 105.2 ($2 \times \text{CH}$), 108.6 ($2 \times \text{C}_q$), 162.0 ($2 \times \text{C}_q$), 163.9 ($2 \times \text{C}_q$), 164.12 ($2 \times \text{C}_q$), 206.5 ($2 \times \text{C}_q$).

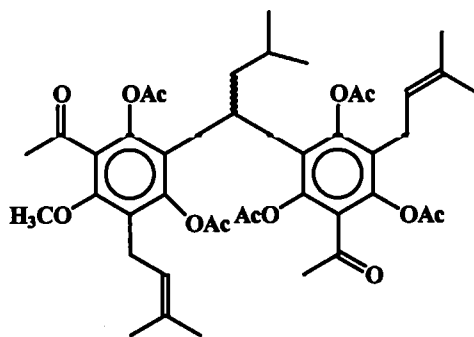
Synthesis of 1,1 di [2,4,6 trimethoxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl]-3-methylbutane (Penta-methoxyacrovestone) (2.58)



Acrovestone (1) (30 mg, 0.05 mmol), dimethyl sulphate (100 mg) and freshly dried K_2CO_3 (0.10 g) were heated to reflux in dry acetone for 15 h. K_2CO_3 was removed by filtration and the filtrate was concentrated *in vacuo*. The crude product was purified by preparative thin layer chromatography using a mixture of diethyl ether:petroleum ether (1:1, v/v) to obtain the desired compound (2.58) (28 mg, 95%) as a yellow oil.

ν_{\max} ($CHCl_3$, cm^{-1}); 1612, 1363, 1175; δ_H ($CDCl_3$); 0.94 (6H, d, J 6.8, $2 \times CH_3$), 1.22 (H, m, $CH(CH_3)_2$), 1.62 (3H, s, CH_3), 1.67 (6H, s, $2 \times CH_3$), 1.72 (3H, s, CH_3), 1.95 (2H, m, CH), 2.52 (3H, s, $2 \times CH_3$), 3.31 (4H, d, J 7.0, $2 \times 2 \times CH_2$), 3.50 (6H, s, $2 \times CH_3$), 3.52 (6H, s, $2 \times CH_3$), 3.60 (6H, s, $2 \times CH_3$), 4.79 (H, t, J 7.0, CH), 5.16 (2H, t, J 7.0, 2H).

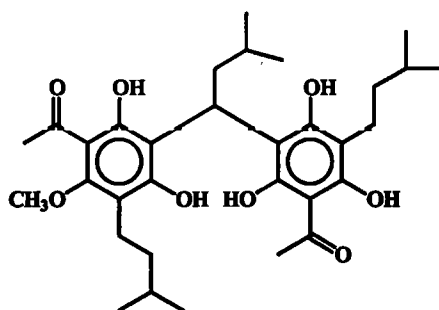
Synthesis of acrovestone penta-acetate (2.49)



Acrovestone (1) (25 mg, 0.04 mmol) was dissolved in a mixture of pyridine (2 ml), acetic anhydride (1 ml) and stirred overnight at room temperature. The reaction mixture was diluted with diethyl ether (20 ml) and washed with 10% aqueous HCl (2 × 10 ml). The organic layer was washed with water (3 × 30 ml), brine, then dried over MgSO₄ and concentrated *in vacuo* to obtain the title compound (2.49) (23 mg, 95%) as a crystalline solid, m.p. 165-166 °C.

ν_{\max} (KBr, cm⁻¹); 1765, 1612, 1585, 1175; δ_{H} (CDCl₃); 0.78 (6H, d, *J* 6.4, 2 × CH₃), 1.24 (1H, m, CH), 1.66 (6H, s, 2 × CH₃), 1.68 (3H, s, CH₃), 1.70 (3H, s, CH₃), 1.95 (2H, m, CH), 2.23 (6H, s, CH₃), 2.26 (3H, s, CH₃), 2.28 (3H, s, CH₃), 2.31 (3H, s, CH₃), 2.40 (3H, s, CH₃), 2.52 (3H, s, CH₃), 3.29 (2H, br s, CH₂), 3.68 (3H, s, CH₃), 4.42 (1H, br s, CH), 5.01 (2H, br s, 2 × CH).

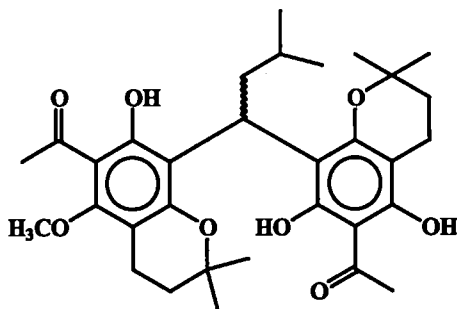
Synthesis of tetrahydroacrovestone (2.50)



A solution of acrovestone (1) (100 mg, 0.17 mmol) in ethyl acetate (20 ml) was stirred under H_2 in the presence of Pd/C (10%) (50 mg) for 3 h at room temperature. The solution was filtered, evaporated *in vacuo* and this resulting crude mixture was purified by dry column flash chromatography using 30% diethyl ether in petroleum ether to yield the title compound (2.50) (90 mg, 90%) as a yellow crystalline solid, m.p. 163-165 °C.

ν_{\max} (KBr, cm^{-1}); 3393, 3246, 2952, 1611, 1461; δ_H ($CDCl_3$); 0.84 (6H, br s, 2 \times CH_3), 0.90 (6H, d, 2 \times CH_3), 0.92 (6H, d, 2 \times CH_3), 1.24 (1H, m, CH), 1.30 (4H, m, 2 \times CH_2), 1.54 (2H, sep, J 7.0, 2 \times CH), 2.14 (2H, m, CH), 2.64 (3H, s, CH_3), 2.66 (3H, s, CH_3), 3.70 (3H, s, OCH_3), 9.04 (1H, br s, OH), 10.12 (1H, br s, OH), 13.21 (1H, br s, OH), 15.40 (1H, br s, OH)

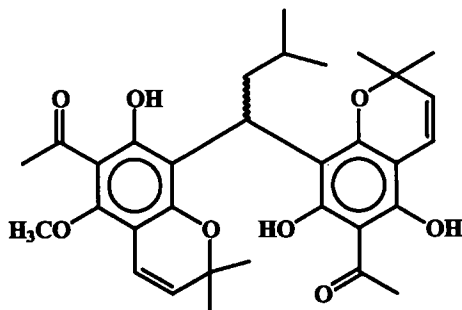
Synthesis of 1-[(2,4-dihydroxy-7,7-dimethyl-chroman-3-oxoethyl-phenyl)-1-(2-hydroxy-4-methoxy-7,7-dimethyl-chroman-3-oxoethyl-phenyl)]-3-methyl butane (2.51)



A solution of acrovestone (1) (0.10 g, 0.1 mmol) in diethyl ether (20 ml) was heated to reflux with Amberlyst 15 (0.10 g) for 6 h. Amberlyst was removed by filtration and the solvent was evaporated *in vacuo*. The resulting gum was subjected to dry column flash chromatography over silica gel using diethyl ether: petroleum ether to obtain the title compound (2.51) (85 mg, 85%) as a yellow semi solid.

ν_{\max} (KBr, cm^{-1}); 3395, 3289, 1612, 1462, 1175; δ_{H} (CDCl_3); 0.86 (6H, br s, 2 \times CH_3), 1.33 (6H, br s, 2 \times CH_3), 1.37 (6H, br s, 2 \times CH_3), 1.40 (1H, m, CH), 2.10 (2H, m, CH_2), 1.80 (4H, t, J 6.5, 2 \times CH_2), 2.58 (4H, t, J 6.5, 2 \times CH_2), 2.65 (3H, s, CH_3), 2.66 (3H, s, CH_3), 3.67 (3H, s, CH_3), 4.81 (1H, t, J 7.4, CH), 9.02 (1H, s, OH), 10.02 (1H, s, OH), 13.02 (1H, s, OH); δ_{C} (CDCl_3); 16.30 (CH_2), 16.40 (CH_2), 22.4 (CH_3), 22.6 (CH_3), 26.8 (2 \times CH_3), 26.9 (2 \times CH_3), 28.8 (CH), 31.4 (2 \times CH_2), 32.2 (CH_3), 33.2 (CH_3), 39.4 (CH_2), 65.4 (CH_3), 106.3 (C_q), 108.2 (C_q), 108.9 (C_q), 116.2 (C_q), 116.8 (C_q), 157.6 (C_q), 161.1 (C_q), 164.5 (C_q), 203.4 (C_q), 203.7 (C_q).

**Synthesis of 1-[(2,4-dihydroxy-7,7-dimethyl-chromen-3-oxoethyl-phenyl)-
1-(2-hydroxy-4-methoxy-7,7-dimethyl-chromen-3-oxoethyl-phenyl)]-3-
methyl butane (2.52)**

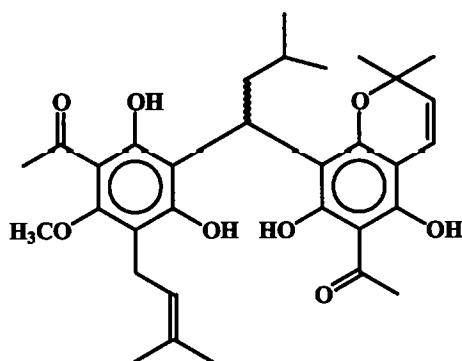


A solution of acrovestone (1) (0.10 g, 0.1 mmol) and DDQ (0.01 g, 0.36 mmol) in dry benzene (20 ml) was heated to reflux for 20 min. The resulting residue was filtered and the filtrate was concentrated *in vacuo*. The crude product was purified by dry column flash chromatography on silica gel using diethyl ether:petroleum ether (3:7, v/v) to afford the title compounds (2.53) (74 mg, 75%) and 2.52 (10 mg 9%) as a yellow semi solids.

ν_{\max} (KBr, cm^{-1}); 3360, 3236, 2972, 1602, 1240, 1230; δ_{H} (CDCl_3); 0.84 (6H, br d, $2 \times \text{CH}_3$), 1.42 (6H, s, $2 \times \text{CH}_3$), 1.68 (3H, s, CH_3), 1.76 (3H, s, CH_3), 2.17 (2H, br s, CH_2), 2.68 (3H, s, CH_3), 2.69 (3H, s, CH_3), 3.22 (2H, br s, CH_2), 3.72 (3H, s, CH_3), 4.74 (1H, t, J , 7.0, CH), 5.42 (1H, d, J 10.0, CH), 5.46 (1H, d, J 10.0, CH), 6.50 (1H, d, J 10.0, CH), 6.63 (1H, d, J 10.0, CH), 9.01 (1H, s, OH), 10.42 (1H, s, OH), 13.43 (1H, s, OH), 15.12 (1H, s, OH); δ_{C} (CDCl_3); 18.0 (CH_3), 22.2 (CH_3), 22.4 (CH_3), 25.4 (CH_3), 28.1 (CH), 30.4 (CH_3), 31.8 (CH_3), 39.6 (CH_2), 55.8 (CH_3), 64.4 (C_q), 64.8 (C_q), 117.06 (CH),

117.38 H), 121.4 (CH), 121.8 (CH), 124.9 (CH), 125.2 (CH), 131.0 (C_q), 160.0 (C_q), 203.1 (C_q), 204.2 (C_q).

1-[(2,4-dihydroxy-7,7-dimethyl-chromen-3-oxoethyl-phenyl)-1-(2-hydroxy-4-methoxy-3-oxoethyl-3-(3-methyl-but-2-enyl)phenyl)]-3-methyl butane (2.53)

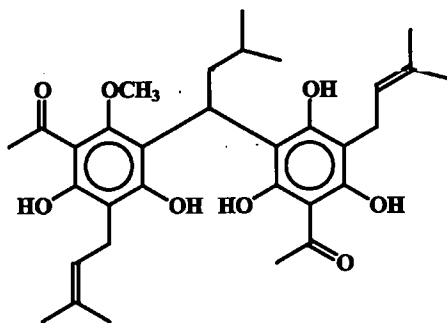


ν_{\max} (KBr, cm⁻¹); 3364, 3246, 2952, 1612, 1232; δ_{H} (CDCl₃); 0.83 (6H, br d, 2 × CH₃), 1.43 (6H, s, 2 × CH₃), 1.68 (3H, s, CH₃), 1.76 (3H, s, CH₃), 2.17 (2H, br s, CH₂), 2.68 (3H, s, CH₃), 2.70 (3H, s, CH₃), 3.29 (2H, br s, CH₂), 3.70 (3H, s, CH₃), 4.72 (1H, t, *J*, 7.6, CH), 5.19 (1H, br s, CH), 5.42 (1H, d, *J* 10.0, CH), 6.63 (1H, d, *J* 10.0, CH), 9.21 (1H, s, OH), 10.01 (1H, s, OH), 13.12 (1H, s, OH), 13.42 (1H, s, OH); δ_{C} (CDCl₃); 17.9 (CH₃), 22.4 (CH₃), 22.6 (CH₃), 25.6 (CH₃), 28.1 (CH), 30.7 (CH₃), 32.8 (CH₃), 40.0 (CH₂), 58.8 (CH₃), 65.8 (C_q), 117.06 (CH), 122.9 (CH), 124.9 (CH), 131.0 (C_q), 160.0 (C_q), 203.1 (C_q), 204.1 (C_q).

General procedure for the synthesis of compounds 2.54- 2.59

A solution of acronylin analogue (2.5, 2.19, 2.20, 2.30, 2.31 and 2.32), demethylacronylin (2.6), isovaleraldehyde and Amberlyst 15 (50 mg) in dichloromethane:diethyl ether (4:1, 10 ml) was heated reflux for 30 min under N₂. Amberlyst was removed by filtration and the filtrate concentrated *in vacuo*. The crude product was purified by dry column flash chromatography using diethyl ether:petroleum ether (3:7, v/v) to afford the title compounds 2.54-2.59.

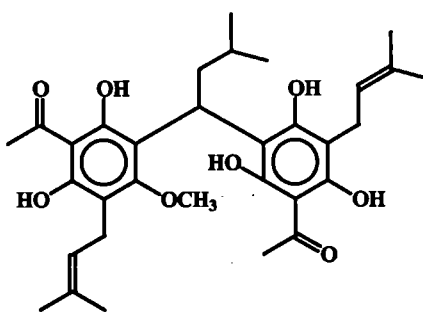
1-[4,6-dihydroxy-2-methoxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl]-1-(2,4,6-trihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl)]-3-methylbutane (2.54)



Following the general procedure compound 2.19 (50 mg, 0.2 mmol) and demethylacronylin (2.6) (47 mg, 0.2 mmol) were condensed with isovaleraldehyde (10 mg, 0.1 mmol) to afford the title compound (2.54) (34 mg, 30%) as a pale yellow crystalline solid, m.p. 140-142 °C.

ν_{\max} (KBr, cm^{-1}); 3396, 3245, 2954, 1608, 1460, 1319; δ_{H} (CDCl_3); 0.84 (6H, s, $\text{CH}(\text{CH}_3)_2$), 1.24 (1H, m, $\text{CH}(\text{CH}_3)_2$), 1.68 (3H, s, CH_3), 1.75 (3H, s, CH_3), 1.77 (3H, s, CH_3), 1.82 (3H, s, CH_3), 2.14 (2H, m, CH_2), 2.49 (3H, s, CH_3), 2.66 (3H, s, CH_3), 3.29 (2H, d, J 7, CH_2), 3.39 (2H, d, J 7, CH_2), 3.70 (3H, OCH_3), 4.74 (1H, m, CH), 5.23 (2H, t, J 7, CH), 6.20 (1H, br s, OH), 9.58 (H, br s, OH), 10.06 (1H, br s, OH), 15.62, (1H, br s, OH), 15.70 (H, br s, OH); δ_{C} (CDCl_3); 17.9 (CH_3), 18.0 (CH_3), 22.3 (CH_2), 22.6 (CH_3), 22.8 (CH_3), 23.1 (CH_2), 25.6 (CH_3), 25.7 (CH_3), 27.0 (CH), 28.8 (CH), 30.6 (CH_3), 32.6 (CH_3), 39.4 (CH_2), 62.6 (CH_3), 104.9 (C_q), 106.3 (C_q), 108.8 (C_q), 108.8 (C_q), 113.2 (C_q), 116.7 (C_q), 121.6 (CH), 123.2 (CH), 131.7 (C_q), 136.7 (C_q), 158.3 (C_q), 158.4 (C_q), 160.3 (C_q), 160.9 (C_q), 204.2 (C_q), 204.3 (C_q).

1-[(2,4-dihydroxy-6-methoxy-3-oxoethyl-5-(3-methyl-but-2-enyl)phenyl)-1-(2,4,6-trihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl)-3-methylbutane (2.55)

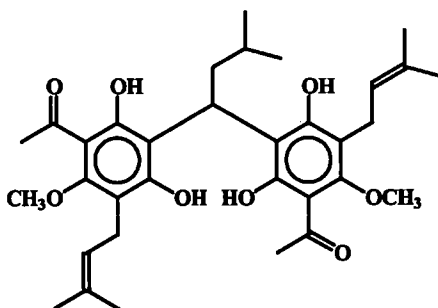


Following the general procedure compound **2.20** (50 mg, 0.2 mmol) and demethylacronylin (**2.6**) (47 mg, 0.2 mmol) were condensed with

isovaleraldehyde (10 mg, 0.1 mmol) to afford the title compound (**2.55**) (30 mg, 30%) as a pale yellow crystalline solid, m.p. 139-142 °C.

ν_{\max} (KBr, cm^{-1}); 3289, 2952, 2920, 1612, 1462, 1363, 1319; δ_{H} (CDCl_3); 0.84 (6H, s, $\text{CH}(\text{CH}_3)_2$), 1.24 (1H, m, $\text{CH}(\text{CH}_3)_2$), 1.75 (6H, s, $2 \times \text{CH}_3$), 1.82 (6H, s, $2 \times \text{CH}_3$), 2.21 (2H, m, CH_2), 2.76 (6H, s, $2 \times \text{CH}_3$), 3.31 (2H, d, J 7, CH_2), 3.39 (2H, d, J 7, CH_2), 3.72 (3H, s, OCH_3), 4.74 (1H, m, CH), 5.21 (2H, t, J 7, CH), 6.21 (1H, br s, OH), 9.58 (H, br s, OH), 10.00 (1H, br s, OH), 15.62, (1H, br s, OH), 15.70 (H, br s, OH); δ_{C} (CDCl_3); 17.9 ($2 \times \text{CH}_3$), 22.3 ($2 \times \text{CH}_2$), 22.5 (CH_3), 22.7 (CH_3), 25.6 ($2 \times \text{CH}_3$), 27.0 (CH), 28.8 (CH), 30.6 ($2 \times \text{CH}_3$), 39.6 (CH_2), 62.6 (CH_3), 104.8 (C_q), 106.2 (C_q), 108.3 (C_q), 108.7 (C_q), 113.2 (C_q), 116.5 (C_q), 121.6 ($2 \times \text{CH}$), 131.6 (C_q), 136.7 (C_q), 158.4 (C_q), 160.3 (C_q), 160.8 (C_q), 160.9 (C_q), 162.6 (C_q), 204.2 ($2 \times \text{C}_q$).

1,1-di-[2,6-dihydroxy-4-methoxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-prenyl]-3-methylbutane (2.56**)**

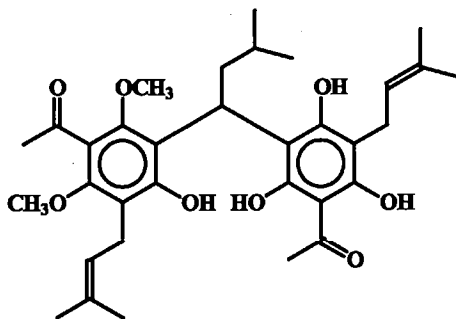


Following the general procedure compound **2.5** (50 mg, 0.2 mmol) was condensed with isovaleraldehyde (10 mg, 0.1 mmol) to afford the title

compound (**2.56**) (25 mg, 22%) as a pale yellow crystalline solid, m.p. 128-130 °C.

ν_{\max} (KBr, cm^{-1}); 3390, 3245, 2952, 1611, 1461, 1363, 1319; δ_{H} (CDCl_3); 0.84 (6H, s, $\text{CH}(\text{CH}_3)_2$), 1.24 (1H, m, $\text{CH}(\text{CH}_3)_2$), 1.76 (6H, s, $2 \times \text{CH}_3$), 1.85 (6H, s, $2 \times \text{CH}_3$), 2.21 (2H, m, CH_2), 2.76 (6H, s, $2 \times \text{CH}_3$), 3.39 (4H, d, J 7, $2 \times \text{CH}_2$), 3.72 (6H, $2 \times \text{OCH}_3$), 4.74 (1H, m, CH), 5.23 (2H, t, J 7, $2 \times \text{CH}$), 6.21 (2H, br s, $2 \times \text{OH}$), 9.58 (2H, br s, $2 \times \text{OH}$), 15.62, (2H, br s, $2 \times \text{OH}$); δ_{C} (CDCl_3); 17.9 ($2 \times \text{CH}_3$), 22.4 ($2 \times \text{CH}_2$), 22.5 (CH_3), 22.7 (CH_3), 25.5 ($2 \times \text{CH}_3$), 27.0 (CH), 28.9 (CH), 31.2 ($2 \times \text{CH}_3$), 39.6 (CH_2), 62.6 ($2 \times \text{CH}_3$), 106.2 ($2 \times \text{C}_q$), 108.2 ($2 \times \text{C}_q$), 108.8 (C_q), 116.5 ($2 \times \text{C}_q$), 121.6 ($2 \times \text{CH}$), 136.6 ($2 \times \text{C}_q$), 160.9 ($2 \times \text{C}_q$), 162.6 ($2 \times \text{C}_q$), 162.8 ($2 \times \text{C}_q$), 204.2 (C_q).

1-(2,4,-dimethoxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-prenyl)-1-(2,4,6-trihydroxy-dihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-prenyl)]-3-methylbutane (2.57**)**

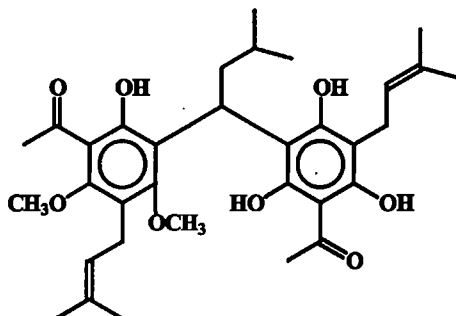


Following the general procedure compound **2.31** (50 mg, 0.18 mmol) and demethylacronylin (**2.6**) (44 mg, 0.18 mmol) were condensed with

isovaleraldehyde (8 mg, 0.1 mmol) to afford the title compound (**2.57**) (26 mg, 25%) as a pale yellow crystalline solid, m.p. 132-132 °C.

ν_{max} (KBr, cm^{-1}); 3245, 2954, 2929, 1615, 1461, 1363, 1320; δ_{H} (CDCl_3); 0.84 (6H, s, $\text{CH}(\text{CH}_3)_2$), 1.24 (1H, m, $\text{CH}(\text{CH}_3)_2$), 1.75 (3H, s, CH_3), 1.76 (3H, s, CH_3), 1.82 (3H, s, CH_3), 1.85 (3H, s, CH_3), 2.21 (2H, m, CH_2), 2.75 (3H, s, CH_3), 2.76 (3H, s, CH_3), 3.31 (2H, d, J 7, CH_2), 3.39 (2H, d, J 7, CH_2), 3.72 (6H, s, $2 \times \text{OCH}_3$), 4.74 (1H, m, CH), 5.21 (2H, t, J 7, $2 \times \text{CH}$), 6.21 (1H, br s, OH), 9.58 (1H, br s, OH), 10.00 (1H, br s, OH), 15.62, (1H, br s, OH); δ_{C} (CDCl_3); 17.9 ($2 \times \text{CH}_3$), 22.3 (CH_2), 22.4 (CH_2), 22.5 (CH_3), 22.7 (CH_3), 25.5 (CH_3), 25.6 (CH_3), 27.0 (CH), 28.9 (CH), 30.6 (CH_3), 32.6 (CH_3), 39.6 (CH_2), 62.6 (OCH_3), 62.8 (OCH_3), 106.2 (C_q), 108.3 ($2 \times \text{C}_q$), 108.2 (C_q), 108.7 (C_q), 113.2 (C_q), 116.5 (C_q), 121.6 ($2 \times \text{CH}$), 136.6 (C_q), 136.7 (C_q), 158.3 (C_q), 158.4 (C_q), 160.2 (C_q), 160.9 (C_q), 162.6 (C_q), 162.8 (C_q), 204.2 ($2 \times \text{C}_q$).

1-(4,6-dimethoxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-prenyl)-1-(2,4,6-trihydroxy-dihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-prenyl)]-3-methylbutane (2.58)



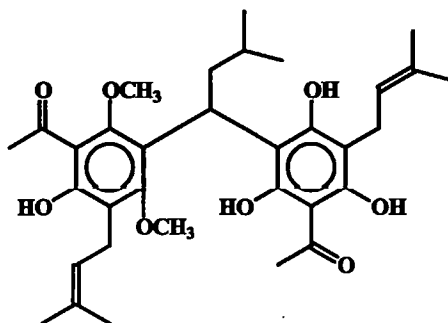
Following the general procedure compound **2.32** (50 mg, 0.18 mmol) and demethylacronylin (**2.6**) (44 mg, 0.18 mmol) were condensed with isovaleraldehyde (8 mg, 0.1 mmol) to afford the title compound (**2.58**) (25 mg, 25%) as a pale yellow crystalline solid, m.p. 138-140 °C.

ν_{\max} (KBr, cm^{-1}); 3260, 2950, 2995, 1619, 1451; δ_{H} (CDCl_3); 0.84 (6H, s, $\text{CH}(\text{CH}_3)_2$), 1.24 (1H, m, $\text{CH}(\text{CH}_3)_2$), 1.75 (3H, s, CH_3), 1.76 (3H, s, CH_3), 1.80 (3H, s, CH_3), 1.84 (3H, s, CH_3), 2.21 (2H, m, CH_2), 2.75 (3H, s, CH_3), 2.75 (3H, s, CH_3), 3.32 (2H, d, J 7, CH_2), 3.39 (2H, d, J 6.8, CH_2), 3.72 (6H, s, $2 \times \text{OCH}_3$), 4.74 (1H, m, CH), 5.21 (2H, t, J 6.8, $2 \times \text{CH}$), 6.21 (1H, br s, OH), 9.58 (H, br s, OH), 10.00 (1H, br s, OH), 15.62, (1H, br s, OH); δ_{C} (CDCl_3); 17.9 ($2 \times \text{CH}_3$), 22.2 (CH_2), 22.4 (CH_2), 22.5 (CH_3), 22.7 (CH_3), 25.5 (CH_3), 25.6 (CH_3), 27.0 (CH), 28.9 (CH), 30.4 (CH_3), 32.5 (CH_3), 39.8 (CH_2), 62.6 (OCH_3), 62.8 (OCH_3), 106.2 (C_q), 108.3 ($2 \times \text{C}_q$), 108.2 (C_q), 108.7 (C_q), 113.2 (C_q), 116.5 (C_q), 121.6 ($2 \times \text{CH}$), 136.6 (C_q), 136.7 (C_q), 158.3 (C_q),

158.4 (C_q), 160.2 (C_q), 160.9 (C_q), 162.6 (C_q), 162.8 (C_q), 204.2 ($2 \times C_q$).

Mass spec. (EI) m/z 584 M^+ .

1-(2,6-dimethoxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-prenyl)-1-(2,4,6-trihydroxy-dihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-prenyl)]-3-methylbutane (2.59)



Following the general procedure compound 2.30 (50 mg, 0.18 mmol) and demethylacronylin (2.6) (44 mg, 0.18 mmol) were condensed with isovaleraldehyde (8 mg, 0.1 mmol) to afford the title compound (2.59) (25 mg, 25%) as a pale yellow crystalline solid, m.p. 139-140 °C.

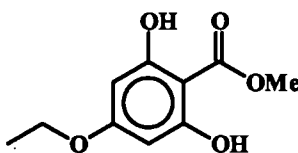
ν_{\max} (KBr, cm^{-1}); 3255, 2954, 2929, 1610, 1461; δ_{H} (CDCl_3); 0.84 (6H, s, $\text{CH}(\text{CH}_3)_2$), 1.24 (1H, m, $\text{CH}(\text{CH}_3)_2$), 1.68 (3H, s, CH_3), 1.75 (3H, s, CH_3), 1.77 (3H, s, CH_3), 1.82 (3H, s, CH_3), 2.21 (2H, m, CH_2), 2.76 (3H, s, $2 \times \text{CH}_3$), 3.29 (2H, d, J 7, CH_2), 3.39 (2H, d, J 7, CH_2), 3.73 (6H, s, $2 \times \text{OCH}_3$), 4.74 (1H, m, CH), 5.21 (2H, t, J 7, $2 \times \text{CH}$), 6.21 (1H, br s, OH), 9.58 (H, br s, OH), 10.00 (1H, br s, OH), 15.12, (1H, br s, OH). 15.62, (1H, br s, OH); δ_{C} (CDCl_3); 17.7 ($2 \times \text{CH}_3$), 22.3 ($2 \times \text{CH}_2$), 22.3 ($2 \times \text{CH}_2$), 22.5 (CH_3), 22.7

(CH₃), 25.5 (CH₃), 25.6 (CH₃), 27.0 (CH), 28.9 (CH), 30.6 (2 × CH₃), 39.6 (CH₂), 62.6 (OCH₃), 62.8 (OCH₃), 106.1 (C_q), 108.8 (2 × C_q), 109.3 (C_q), 113.2 (C_q), 116.4 (C_q), 121.6 (2 × CH), 136.6 (C_q), 136.7 (C_q), 158.3 (C_q), 158.4 (C_q), 160.3 (C_q), 160.9 (C_q), 162.6 (C_q), 162.8 (C_q), 204.2 (2 × C_q).

General procedure for the synthesis of compounds 2.61-2.64

To an ice cold solution of methylbenzoate (1 equivalent) and dry alcohol (1.01 equivalent) in dry THF (50 ml) were added PPh₃ (1 equivalent) and DEAD (1 equivalent). The mixture was warmed to room temperature over 2 h. The solution was diluted with diethyl ether (50 ml), washed with water (3 × 50 ml), dried over MgSO₄ and concentrated *in vacuo*. The crude product was dry column chromatographed on silica gel using a mixture of diethyl ether:petroleum ether (2:3, v/v) to afford the title compounds, 2.61-2.64.

Synthesis of methyl-4-ethoxy-2,6-dihydroxy-benzoate (2.61)

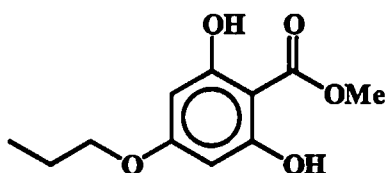


Following the general procedure methylbenzoate (2.60) (3.5 g, 19.0 mmol), ethanol (1.0 g, 21 mmol), PPh₃ (5.0 g, 19 mmol) and DEAD (3.3 g, 19 mmol) were reacted to afford the title compound (2.61) (3.10 g, 78%), as a white crystalline solid, m.p. 144-145 °C.

ν_{\max} (KBr, cm^{-1}); 3240, 3027, 3000, 2943, 1680, 1635, 1580, 1500, 1358;

δ_{H} (CDCl_3); 1.52 (3H, t, J 6.8, CH_3), 4.13 (2H, q, J 6.8, CH_2), 4.00 (3H, s, OCH_3), 5.93 (2H, s, Ar-H), 9.42 (1H, s, OH), 14.81 (1H, s, OH); δ_{C} (CDCl_3); 14.4 (CH_3), 52.5 (CH_3), 65.5 (CH_2), 91.7 ($2 \times \text{CH}$), 95.2 (C_q), 166.0 (C_q), 170.0 (C_q), 170.3 (C_q), 171.5 (C_q).

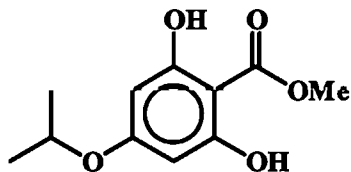
Methyl-4-propoxy-2,6-dihydroxybenzoate (2.62)



Following the general procedure methylbenzoate (2.60) (3.5 g, 19.0 mmol), propanol (1.5 g, 24 mmol), PPh_3 (5.0 g, 19 mmol) and DEAD (3.3 g, 19 mmol) were reacted to afford the title compound (2.62) (3.00 g, 70%) as a white crystalline solid, m.p. 60-62 °C.

ν_{\max} (KBr, cm^{-1}); 3412, 1640, 1582, 1345; δ_{H} (CDCl_3); 0.97 (3H, dd, J 14.1, 7.3, CH_3), 1.73 (2H, m, J 14.1, 6.8, CH_2), 3.93 (2H, d, J 6.8, 2H), 4.06 (3H, s, OCH_3), 6.08 (2H, s, Ar-H), 9.40 (1H, s, OH), 14.51 (1H, s, OH); δ_{C} (CDCl_3); 10.5 (CH_3), 22.9 (CH_2), 52.3 (3H, CH_3), 70.1 (3H, s, CH_2), 94.9 ($2 \times \text{C}_q$), 106.4 (C_q), 164.2 (C_q), 165.7 ($2 \times \text{C}_q$), 169.5 (C_q). Mass spec. (EI) m/z 226 M^+ .

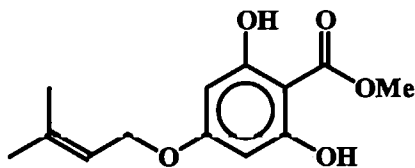
Methyl-4-isopropoxy-2,6-dihydroxybenzoate (2.63)



Following the general procedure methylbenzoate (**2.60**) (3.5 g, 19.0 mmol), isopropanol (1.5 g, 24 mmol), PPh_3 (5.0 g, 19 mmol) and DEAD (3.3 g, 19 mmol) were reacted to afford the title compound (**2.63**) (2.5 g, 47%) as a white crystalline solid, m.p. 53-54 °C.

ν_{max} (KBr, cm^{-1}); 3225, 3027, 1650, 1431, 1314; δ_{H} (CDCl_3); 1.32 (3H, s, CH_3), 1.33 (3H, s, CH_3), 4.54 (1H, m, H), 4.01 (3H, s, OCH_3), 6.00 (2H, s, Ar-H), 9.82 (1H, s, OH), 14.01 (1H, s, OH); δ_{C} (CDCl_3); 21.9 ($2 \times \text{CH}_3$), 52.4 (CH_3), 70.2 (CH), 93.5 (CH), 95.5 ($2 \times \text{C}_q$), 163.2 (C_q), 164.9 ($2 \times \text{C}_q$) 169.9 (C_q).

Methyl-4-isopentenyl-2,6-dihydroxybenzoate (2.64)



Following the general procedure methylbenzoate **2.60** (3.5 g, 19.0 mmol), 3-methyl-but-2-ene-ol (1.8 g, 20 mmol), PPh_3 (5.0 g, 19 mmol) and DEAD (3.3

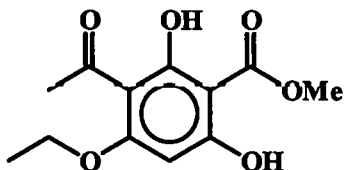
g, 19 mmol) were reacted to afford the title compound (**2.64**) (3.5 g, 75%) as a white crystalline solid, m.p. 90-92 °C.

ν_{max} (KBr, cm^{-1}); 3440, 2962, 1662, 1577, 1260, 1157; δ_{H} (CDCl_3); 1.73 (3H, s, CH_3), 1.79 (3H, s, CH_3), 4.02 (3H, s, OCH_3), 4.50 (2H, d, J 6.3, CH_2), 5.44 (H, t, J 6.3, CH), 6.03 (2H, s, Ar-H), 9.82 (1H, s, OH), 14.01 (1H, s, OH); δ_{C} (CDCl_3); 18.4 (CH_3), 26.0 (CH_3), 52.7 (CH_3), 65.2 (CH_2), 95.2 (CH), 106.7 (C_q), 162.0 (C_q), 165.9 ($2 \times \text{C}_q$), 169.8 (C_q).

General procedure for the synthesis of compounds **2.65-2.68**

To an ice cold solution of TiCl_4 (1 equivalent) and AcCl (1 equivalent) was added methylbenzoate (**2.61-2.64**) (1 equivalent) in dry benzene (20 ml) under N_2 . The mixture was stirred at room temperature for 20 min and added 2N HCl (20 ml). The organic layer was diluted with diethyl ether (25 ml), washed with saturated aqueous solution of NaHCO_3 , brine, water, dried over MgSO_4 , and concentrated *in vacuo* to obtain a gummy residue. The residue was purified by column chromatography on silica gel using a mixture of diethyl ether:petroleum ether (2:3, v/v) to yield the title compounds **2.65-2.68**.

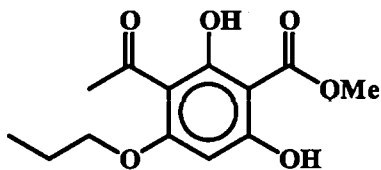
Methyl-4-ethoxy-3-oxoethyl-2,6-dihydroxybenzoate (2.65)



Following the general procedure compound 2.61 (2.5 g, 11 mmol) was acylated using TiCl_4 (2.23 ml, 11 mmol) and AcCl (1.0 g, 11 mmol) to afford title compound (2.65) (2.5 g, 83%) as a white crystalline solid, m.p.138-139 °C.

ν_{max} (KBr, cm^{-1}); 3370, 3065, 2962, 1682, 1577, 1259; δ_{H} (CDCl_3); 1.52 (3H, t, J 6.8, CH_3), 2.64 (3H, s, CH_3), 4.14 (2H, q, J 6.8, CH_2), 3.97 (3H, s, OCH_3), 6.03 (1H, s, Ar-H), 9.42 (1H, s, OH), 14.81 (1H, s, OH); δ_{C} (CDCl_3); 14.5 (CH_3), 32.2 (CH_3), 52.5 (CH_3), 65.5 (CH_2), 91.7 (CH), 95.0 (C_q), 104.8 (C_q), 166.0 (C_q), 170.0 (C_q), 170.3 (C_q), 171.7 (C_q).

Methyl-2,6-dihydroxy-4-propoxy-3-oxoethyl-dihydroxybenzoate (2.66)

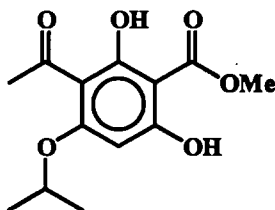


Following the general procedure compound 2.62 (2.5 g, 11 mmol) was acylated using TiCl_4 (2.2 ml, 11 mmol) and AcCl (1.0 g, 11 mmol) to afford

the title compound (**2.66**) (2.6 g, 86%) as a white crystalline solid, m.p.80-81 °C.

ν_{\max} (KBr, cm^{-1}); 3446, 2955, 1652, 1642, 1558; δ_{H} (CDCl_3); 0.95 (3H, dd, J 14.6, 7.0, CH_3), 1.78 (2H, m, J 14.1, 6.8, CH_2), 2.61 (CH_3), 3.94 (2H, d, J 6.8, 2H), 4.06 (3H, s, OCH_3), 5.91 (H, s, Ar), 12.21 (1H, s, OH), 14.51 (1H, s, OH); δ_{C} (CDCl_3); 10.2 (CH_3), 23.0 (CH_2), 33.2 (CH_3), 52.3 (3H, OCH_3), 70.1 (3H, s, CH_2), 94.9 (C_q), 104.2 (C_q), 106.4 (C_q), 164.3 (C_q), 165.8 ($2 \times \text{C}_q$), 169.5 (C_q), 203.3 (C_q).

Methyl-2,6-dihydroxy-4-isopropoxy-3-oxoethyl-dihydroxybenzoate (2.67)

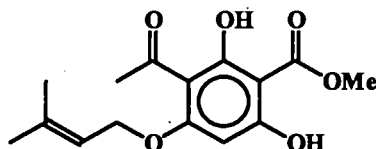


Following the general procedure compound **2.63** (2.5 g, 11 mmol) was acylated using TiCl_4 (2.2 ml, 11 mmol) and AcCl (1.0 g, 11 mmol) to afford the title compound (**2.67**) (1.5 g, 50%) as a white crystalline solid, m.p.67-68 °C.

ν_{\max} (KBr, cm^{-1}); 3370, 3065, 2962, 1682, 1577, 1314; δ_{H} (CDCl_3); 1.32 (3H, s, CH_3), 1.31 (3H, s, CH_3), 2.63 (3H, s, CH_3), 4.53 (1H, m, CH), 4.02 (3H, s, OCH_3), 6.00 (1H, s, Ar), 12.01 (1H, s, OH), 14.01 (1H, s, OH); δ_{C} (CDCl_3);

21.9 (2 × CH₃), 52.4 (CH₃), 70.1 (CH), 93.5 (CH), 95.5 (C_q), 104.2 (C_q), 163.2 (C_q), 164.9 (2 × C_q), 169.9 (C_q), 203.2 (C_q).

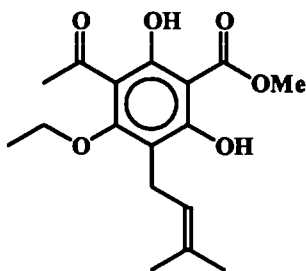
Methyl-2,6-dihydroxy-4-(3-methyl-but-2-enyloxy)-3-oxoethyl-dihydroxybenzoate (2.68)



Following the general procedure compound **2.64** (2.5 g, 11 mmol) was acylated using TiCl₄ (1.8 ml, 10 mmol) and AcCl (1.0 g, 11 mmol) to afford the title compound (**2.68**) (2.7 g, 93%) as a white crystalline solid, m.p. 72-73 °C.

ν_{\max} (KBr, cm⁻¹); 3414, 3095, 2990, 1698, 1639, 1256, 1106; δ_{H} (CDCl₃); 1.73 (3H, s, CH₃), 1.79 (3H, s, CH₃), 2.62 (3H, s, CH₃), 4.02 (3H, s, OCH₃), 4.50 (2H, d, *J* 6.3, CH₂), 5.44 (1H, t, *J* 6.3, CH), 6.03 (1H, s, Ar), 12.82 (1H, s, OH), 14.01 (1H, s, OH); δ_{C} (CDCl₃); 18.4 (CH₃), 26.0 (CH₃), 52.7 (CH₃), 65.2 (CH₂), 95.2 (CH), 104.2 (C_q), 106.7 (C_q), 162.0 (C_q), 165.9 (2 × C_q), 169.8 (C_q), 203.4 (C_q).

Synthesis of methyl-2,6-dihydroxy-4-ethoxy-3-oxoethyl-5-(3-methyl-but-2-enyl) benzoate (2.69)

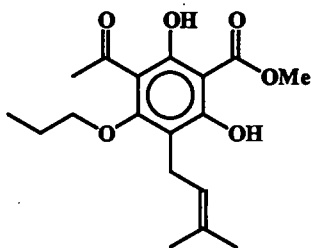


To a solution of compound (2.65) (2.0 g, 7.8 mmol) in absolute dioxane (35 ml) $\text{BF}_3\text{-Et}_2\text{O}$ (2 ml) was added slowly. The reaction mixture was warmed to 40°C and stirred for 30 min. A solution of 2-methyl-but-3-ene-2-ol (1.0 g, 11 mmol) in dry dioxane (7 ml) was added to the mixture and stirred for another 2 h. The reaction mixture was cooled to room temperature, diluted with diethyl ether (50 ml) and washed with water (3×50 ml). The organic extracts were dried over MgSO_4 and concentrated *in vacuo*. The resulting gum was purified by column chromatography on silica gel using diethyl ether:petroleum ether as an eluent (1:9, v/v) to obtain the desired product (2.69) (300 mg, 12%) as a white crystalline solid, m.p. $110\text{-}112^\circ\text{C}$.

ν_{max} (KBr, cm^{-1}); 3360, 3040, 2900, 1682, 1557, 1250; δ_{H} (CDCl_3); 1.52 (3H, t, J 6.8, CH_3), 1.65 (3H, s, CH_3), 1.76 (3H, s, CH_3), 2.58 (3H, s, CH_3), 3.26 (2H, d, J 6.8, CH_2), 4.01 (2H, q, J 6.8, CH_2), 4.01 (3H, s, OCH_3), 5.18 (1H, t J 6.8 CH), 9.42 (1H, s, OH), 14.81 (1H, s, OH); δ_{C} (CDCl_3); 14.4 (CH_3), 17.7 (CH_3), 21.2 (CH_2), 25.8 (CH_3), 33.3 (CH_3), 50.6 (CH_3), 65.5 (CH_2), 95.0 (C_q),

104.8 (C_q), 122.7 (CH), 131.3 (C_q), 166.0 (C_q), 170.0 (C_q), 170.3 (C_q), 171.7 (C_q), 203.2 (C_q).

Synthesis of methyl-2,6-dihydroxy-4-propoxy-3-oxoethyl-5-(3-methyl-but-2-enyl) benzoate (2.70)

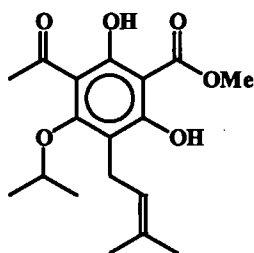


To a solution of compound (2.66) (2.5 g, 7.5 mmol) in absolute dioxane (10 ml) BF₃-Et₂O (2 ml) was added slowly. The reaction mixture was warmed to 40 °C and stirred for 30 min. A solution of 2-methyl-but-3-ene-2-ol (1.0 g, 11 mmol) in dry dioxane (7 ml) was added to the mixture and stirred for another 2 h. The reaction mixture was cooled to room temperature, diluted with diethyl ether (50 ml), washed with water (3 × 50 ml), then dried over MgSO₄ and concentrated *in vacuo*. The resulted gum was column chromatographed on silica gel using diethyl ether:petroleum ether as an eluent (1:9, v/v) to obtain the desired product (2.70) (300 mg, 12%) as a white crystalline solid, m.p. 84-85 °C.

ν_{max} (KBr, cm⁻¹); 3440, 2940, 1640, 1642, 1550; δ_{H} (CDCl₃); 0.97 (3H, dd, *J* 14.6, 7.0, CH₃), 1.72 (3H, s, CH₃), 1.73 (2H, m, *J* 14.1, 6.8, CH₂), 1.75 (3H, s, CH₃), 2.61 (3H, s, CH₃), 3.32 (2H, d, *J* 6.8, CH₂), 3.95 (2H, dd, *J* 6.8, CH₂),

4.06 (3H, s, OCH₃), 5.2 (H, t, *J*, 6.8, CH), 12.21 (1H, s, OH), 14.51 (1H, s, OH); δ_c (CDCl₃); 10.3 (CH₃), 17.5 (CH₃), 22.3 (CH₂), 23.0 (CH₂), 26.2 (CH₃), 33.2 (CH₃), 52.3 (CH₃), 70.1 (CH₂), 94.9 (C_q), 104.2 (C_q), 106.2 (C_q), 106.6 (C_q), 121.6 (CH), 136.2 (C_q), 164.5 (C_q), 165.2 (2 × C_q), 165.4 (2 × C_q), 169.5 (C_q), 203.3 (C_q).

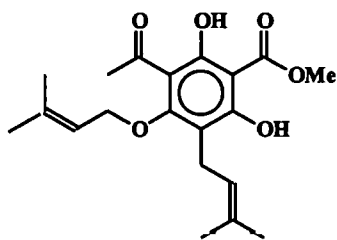
Synthesis of methyl-2,6-dihydroxy-4-isopropoxy-3-oxoethyl-5-(3-methyl-but-2-enyl) benzoate (2.71)



To a solution of compound (2.67) (1.5 g, 5.5 mmol) in absolute dioxane (35 ml) BF₃-Et₂O (1 ml) was added slowly. The reaction mixture was warmed to 40 °C and stirred for 30 min. A solution of 2-methyl-but-3-ene-2-ol (0.8 g, 9 mmol) in dry dioxane (2 ml) was added to the mixture and stirred for another 2 h. The reaction mixture was cooled to room temperature, diluted with diethyl ether (50 ml) and washed with water (3 × 50 ml). The organic extract was dried over MgSO₄ and concentrated *in vacuo*. The resulting gum was purified by column chromatography on silica gel using a mixture of diethyl ether:petroleum ether (1:9, v/v) to obtain the desired product (2.71) (225 mg, 12%) as a white crystalline solid, m.p. 80-81 °C.

ν_{\max} (KBr, cm^{-1}); 3412, 1640, 1634, 1598, 1150; δ_{H} (CDCl_3); 1.32 (3H, s, CH_3), 1.31 (3H, s, CH_3), 1.76 (3H, s, CH_3), 1.79 (3H, s, CH_3), 2.63 (3H, s, CH_3), 3.39 (2H, d, J 7, CH_2), 3.92 (3H, s, OCH_3), 4.50 (1H, m, H), 5.21 (1H, d, J 7, CH), 12.01 (H, s, OH), 14.01 (H, s, OH); δ_{C} (CDCl_3); 17.9 (CH_3), 21.9 ($2 \times \text{CH}_3$), 22.3 (CH_2), 25.2 (CH_3), 52.4 (CH_3), 70.1 (CH), 93.5 (CH), 104.0 (C_q), 106.2 (C_q), 122.0 (CH), 131.3 (C_q), 163.2 (C_q), 164.9 ($2 \times \text{C}_q$), 169.9 (C_q), 203.2 (C_q).

Synthesis of methyl-2,6-dihydroxy-4-(3-methyl-but-2-enyloxy)-3-oxoethyl-5-(3-methyl-but-2-enyl) benzoate (2.72)

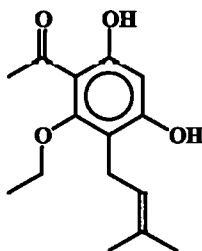


To a solution of compound (2.68) (2.0 g, 6.8 mmol) in absolute dioxane (15 ml) $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (2 ml) was added slowly. The reaction mixture was warmed to 40°C and stirred for 30 min. A solution of 2-methyl-but-3-ene-2-ol (0.8 g, 9.3 mmol) in dry dioxane (4 ml) was added to the mixture and stirred for another 2 h. The reaction mixture was cooled to room temperature, diluted with diethyl ether (50 ml) and washed with water (3×50 ml). The organic extract was dried over MgSO_4 and concentrated *in vacuo*. The resulting gum was purified by column chromatography on silica gel using a mixture of diethyl

ether:petroleum ether (1:9, v/v) to obtain the title compound (**2.72**) (295 mg, 12%) as a yellow crystalline solid, m.p. 70-71 °C.

ν_{\max} (KBr, cm^{-1}); 3440, 3335, 1634, 1370; δ_{H} (CDCl_3); 1.68 (3H, s, CH_3), 1.73 (3H, s, CH_3), 1.75 (3H, s, CH_3), 1.79 (3H, s, CH_3), 3.29 (2H, d, J 7, CH_2), 4.02 (3H, s, OCH_3), 4.50 (2H, d, J 6.3, CH_2), 5.21 (1H, t, J 7, CH), 5.44 (1H, t, J 6.3, CH), 12.82 (1H, s, OH), 14.01 (1H, s, OH); δ_{C} (CDCl_3); 17.9 (CH_3), 18.4 (CH_3), 22.3 (CH_2), 25.6 (CH_3), 26.0 (CH_3), 52.7 (CH_3), 65.2 (CH_2), 104.6 (C_q), 106.3 (C_q), 119.2 (CH), 121.6 (CH), 136.7 (C_q), 139.2 (C_q), 162.1 (C_q), 164.5 ($2 \times \text{C}_q$), 169.8 (C_q), 203.4 (C_q).

Synthesis of 4,6-dihydroxy-2-ethoxy-3-(3-methyl-but-2-enyl)-phenyl-ethanone (2.73**)**

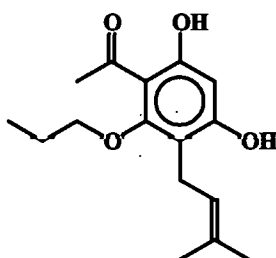


The compound **2.69** (200 mg, 0.62 mmol), 50% aqueous KOH (5 ml) and DMSO (10 ml) were heated to 115 °C for 2 h. On cooling, 10% HCl (20 ml) was added and the product extracted with diethyl ether (3×50 ml). The combined extracts were washed with water (3×50 ml), dried over MgSO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography over silica gel using a mixture of diethyl ether:petroleum

ether (1:1, v/v) to afford the title compound (**2.73**) (114 mg, 75%) as a crystalline solid, m.p. 100-101 °C.

ν_{max} (KBr, cm^{-1}); 3440, 1650, 1400; δ_{H} (CDCl_3); 1.41 (3H, t, J 6.8, CH_3), 1.71 (3H, s, CH_3), 1.78 (3H, s, CH_3), 2.67 (3H, s, CH_3), 3.26 (2H, d, J 6.8, CH_2), 4.05 (2H, q, J 6.8, CH_2), 5.18 (1H, t, J 6.8, CH), 5.90 (1H, s, Ar), 12.42 (1H, s, OH), 14.81 (1H, s, OH); δ_{C} (CDCl_3); 14.5 (CH_3), 17.7 (CH_3), 21.2 (CH_2), 25.8 (CH_3), 33.3 (CH_3), 64.2 (CH_2), 85.7 (CH), 105.8 (C_q), 109.5 (C_q), 122.7 (CH), 131.3 (C_q), 161.7 (C_q), 163.2 (C_q), 163.4 (C_q), 203.2 (C_q).

Synthesis of 4,6-dihydroxy-2-propoxy-3-(3-methyl-but-2-enyl)-phenyl-ethanone (2.74**)**

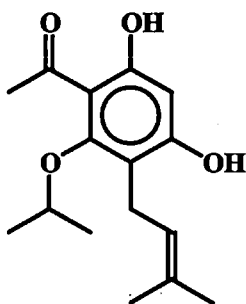


The compound **2.70** (200 mg, 0.62 mmol), 50% aqueous KOH (5 ml) and DMSO (10 ml) were heated to 115 °C for 2 h. On cooling, 10% HCl (20 ml) was added and the product extracted with diethyl ether (3 × 50 ml). The combined extracts were washed with water (3 × 50 ml), dried over MgSO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography over silica gel using a mixture of diethyl ether:petroleum

ether (1:1, v/v) to afford the title compound (**2.74**) (114 mg, 75%) as a crystalline solid, m.p. 88-89 °C.

ν_{max} (KBr, cm^{-1}); 3269, 1620, 1420, 1122; δ_{H} (CDCl_3); 0.97 (3H, dd, J 14.6, 7.0, CH_3), 1.72 (3H, s, CH_3), 1.73 (2H, m, J 14.1, 6.8, CH_2), 1.82 (3H, s, CH_3), 2.62 (3H, s, CH_3), 3.32 (2H, d J 6.8, CH_2), 3.93 (2H, d, J 6.8, CH_2), 5.2 (H, t, J , 6.8, CH), 6.01 (1H, s, Ar), 12.21 (1H, s, OH), 14.51 (1H, s, OH); δ_{C} (CDCl_3); 10.5 (CH_3), 17.5 (CH_3), 22.3 (CH_2), 23.0 (CH_2), 26.2 (CH_3), 33.2 (CH_3), 70.2 (3H, s, CH_2), 95.3 (C_q), 105.0 (C_q), 106.3 (C_q), 121.6 (CH), 136.2 (C_q), 164.2 (C_q), 165.7 ($2 \times \text{C}_q$), 203.3 (C_q).

Synthesis of 4,6-dihydroxy-2-isopropoxy-3-(3-methyl-but-2-enyl)-phenyl-ethanone (**2.75**)

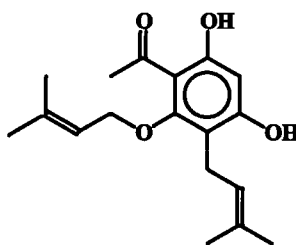


The compound **2.71** (200 mg, 0.62 mmol), 50% aqueous KOH (5 ml) and DMSO (10 ml) were heated to 115 °C for 2 h. On cooling, 10% HCl (20 ml) was added and the product extracted with diethyl ether (3 \times 50 ml). The combined extracts were washed with water (3 \times 50 ml), dried over MgSO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography over silica gel using a mixture of diethyl ether:petroleum

ether (1:1, v/v) to afford the title compound (**2.75**) (114 mg, 75%), as a crystalline solid, m.p. 94-96 °C.

ν_{max} (KBr, cm^{-1}); 3133, 1595, 1420, 1214; δ_{H} (CDCl_3); 1.32 (3H, s, CH_3), 1.33 (3H, s, CH_3), 1.62 (3H, s, CH_3), 1.73 (3H, s, CH_3), 2.61 (3H, s, CH_3), 3.24 (2H, d, J 7, CH_2), 4.54 (1H, m, H), 5.22 (1H, d, J 7, CH), 6.01 (1H, s, Ar), 12.01 (1H, s, OH), 14.01 (1H, s, OH); δ_{C} (CDCl_3); 17.8 (CH_3), 21.9 ($2 \times \text{CH}_3$), 21.8 (CH_2), 25.8 (CH_3), 33.1 (CH_3), 70.1 (CH), 94.8 (CH), 105.8 (C_q), 109.5 (C_q), 122.0 (CH), 131.3 (C_q), 161.6 (C_q), 163.4 (C_q), 163.6 (C_q), 203.2 (C_q).

Synthesis of 4,6-dihydroxy-2-(3-methyl-but-2-enyloxy)-3-(3-methyl-but-2-enyl)-phenyl-ethanone (2.76**)**



The compound **2.72** (200 mg, 0.55 mmol), 50% aqueous KOH (5 ml) and DMSO (10 ml) were heated to 115 °C for 2 h. On cooling, 10% HCl (20 ml) was added and the product extracted with diethyl ether (3 \times 50 ml). The combined extracts were washed with water (3 \times 50 ml), dried over MgSO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography over silica gel using a mixture of diethyl ether:petroleum

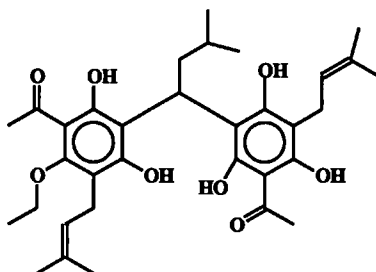
ether (1:1, v/v) to afford the title compound (**2.76**) (117 mg, 70%) as a crystalline solid, m.p. 71-72 °C.

ν_{max} (KBr, cm^{-1}); 3345, 1645, 1085, 835; δ_{H} (CDCl_3); 1.69 (3H, s, CH_3), 1.73 (3H, s, CH_3), 1.76 (3H, s, CH_3), 1.79 (3H, s, CH_3), 2.67 (3H, s, CH_3), 3.28 (2H, d, J 7, CH_2), 4.50 (2H, d, J 6.3, CH_2), 5.21 (1H, t, J 7, CH), 5.44 (1H, t, J 6.3, CH), 6.01 (1H, s, Ar), 12.82 (1H, s, OH), 14.01 (1H, s, OH); δ_{C} (CDCl_3); 17.9 (CH_3), 18.4 (CH_3), 22.3 (CH_2), 25.6 (CH_3), 26.0 (CH_3), 65.2 (CH_2), 95.6 (CH), 104.8 (C_q), 119.2 (CH), 121.6 (CH), 136.7 (C_q), 139.2 (C_q), 162.1 (C_q), 164.5 ($2 \times \text{C}_q$), 203.4 (C_q).

General procedure for the synthesis of compounds **2.77-2.80**

A solution of acronylin analogues (**2.73-2.76**), demethylacronylin (**2.6**), isovaleraldehyde and Amberlyst 15 (50 mg) in a mixture of dichloromethane:diethyl ether (4:1, 10 ml) were heated to reflux for 30 min under N_2 . Amberlyst was removed by filtration and the filtrate concentrated *in vacuo*. The crude product was purified by dry column flash chromatography using a mixture of diethyl ether:petroleum ether (3:7, v/v) to afford the title compounds **2.77-2.80**.

1-[(2,6-dihydroxy-4-ethoxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl)-1-(2,4,6-trihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl)]-3-methylbutane (2.77)

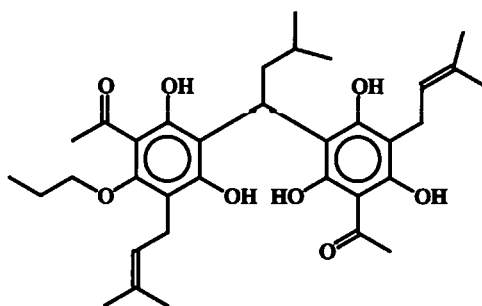


Following the general procedure the compound **2.73** (50 mg, 0.18 mmol), demethylacronylin (**2.6**) (44 mg, 0.18 mmol) and isovaleraldehyde (8 mg, 0.9 mmol) were condensed to afford the title compound (**2.77**) (37 mg, 35%) as a pale yellow crystalline solid, m.p. 120-121 °C.

ν_{\max} (KBr, cm^{-1}); 3412, 3095, 2990, 2980, 1716, 1636, 1437, 1193, 1026; δ_{H} (CDCl_3); 0.87 (6H, br s, $2 \times \text{CH}_3$), 1.40 (1H, m, CH), 1.52 (3H, t, J 6.8, CH_3), 1.69 (3H, s, CH_3), 1.76 (6H, s, CH_3), 1.78 (3H, s, CH_3), 1.84 (3H, s, CH_3), 2.12 (2H, m), 2.67 (3H, s, CH_3), 2.70 (3H, s, CH_3), 3.47 (2H, d, J 7, CH_2), 3.48 (2H, d, J 7.0, CH_2), 4.14 (2H, q, J 6.8, OCH_2), 4.74 (1H, t, J 7.7, CH), 5.21 (2H, t, J 7.0, $2 \times \text{CH}$), 6.22 (1H, br s, OH), 9.50 (1H, br s, OH), 10.06 (1H, br s, OH), 15.62, (1H, br s, OH), 15.70 (1H, br s, OH); δ_{C} (CDCl_3); 14.5 (CH_3), 17.9 (CH_3), 18.0 (CH_3), 22.3 (CH_2), 22.4 (CH_3), 22.6 (CH_3), 23.1 (CH_2), 23.2 (CH_2), 25.6 (CH_3), 25.7 (CH_3), 27.0 (CH), 28.8 (CH), 30.5 (CH_3), 32.5 (CH_3), 39.4 (CH_2), 65.5 (CH_2), 106.3 (C_q), 106.7 (C_q), 108.2 ($2 \times \text{C}_q$),

113.1 (C_q), 116.7 (C_q), 121.7 (CH), 123.2 (CH), 131.6 (C_q), 136.6 (C_q), 160.0 (C_q), 160.2 (C_q), 160.6 (C_q), 160.7 (C_q), 160.8 (C_q), 162.6 (C_q), 204.1 (C_q), 204.3 (C_q).

1-[(2,6-dihydroxy-4-propoxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl)-1-(2,4,6-trihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl)]-3-methylbutane (2.78)

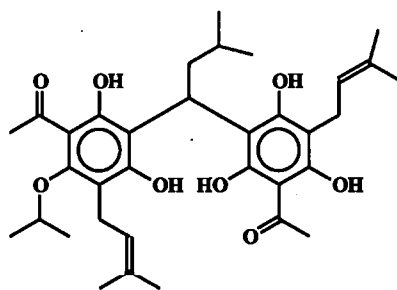


Following the general procedure the compound **2.74** (50 mg, 0.17 mmol), demethylacronylin (**2.6**) (42 mg, 0.17 mmol) and isovaleraldehyde (8 mg, 0.09 mmol) were condensed to afford the title compound (**2.78**) (36 mg, 35%) as a pale yellow crystalline solid, m.p. 128-129 °C.

ν_{max} (KBr, cm⁻¹); 3396, 3246, 2954, 2929, 1608, 1460, 1360, 1175, 1098; δ_{H} (CDCl₃); 0.87 (6H, br s, 2 × CH₃), 0.98 (3H, dd, *J* 7.3, 14.5, CH₃), 1.41 (1H, m, CH), 1.68 (3H, s, CH₃), 1.69 (3H, s, CH₃), 1.73 (2H, m, *J* 7.3, 14.5, CH₃), 1.76 (3H, s, CH₃), 1.83 (3H, s, CH₃), 2.12 (2H, m, CH₂), 2.66 (3H, s, CH₃), 2.70 (3H, s, CH₃), 3.39 (2H, d, *J* 6.6, CH₂), 3.41 (2H, d, *J* 6.6, CH₂), 3.93 (3H, t, *J* 6.8, OCH₂), 4.75 (H, t, *J* 7.7, CH), 5.20 (2H, *J* 6.6, 2 × CH), 6.20 (1H, br s, OH), 9.58 (1H, br s, OH), 10.06 (1H, br s, OH), 15.62, (1H, br s, OH), 15.70

(1H, br s, OH); δ_C (CDCl₃); 10.5 (CH₃), 17.9 (CH₃), 18.0 (CH₃), 22.2 (CH₃), 22.3 (CH₂), 22.5 (CH₃), 23.2 (CH₂), 25.6 (CH₃), 25.8 (CH₃), 26.9 (CH), 28.5 (CH), 22.9 (CH₂), 30.6 (CH₃), 32.1 (CH₃), 39.3 (CH₂), 70.1 (CH₂), 106.2 (C_q), 106.3 (C_q), 108.2 (2 × C_q), 113.1 (C_q), 116.6 (C_q), 121.5 (CH), 123.0 (CH), 131.7 (C_q), 136.7 (C_q), 158.0 (C_q), 158.2 (C_q), 160.2 (C_q), 160.5 (C_q), 160.7 (C_q), 162.7 (C_q), 204.2 (C_q), 204.3 (C_q).

Synthesis of 1-[(2,6-dihydroxy-4-isopropoxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl)-1-(2,4,6-trihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl)]-3-methylbutane (2.79)

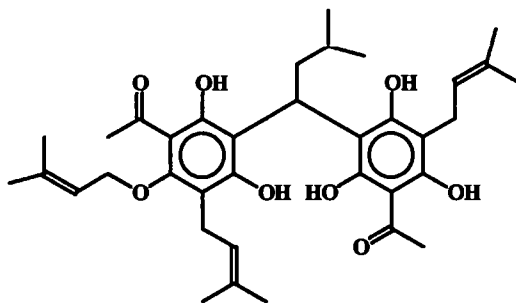


Following the general procedure the compound **2.75** (50 mg, 0.17 mmol), demethylacronylin (**2.6**) (42 mg, 0.17 mmol) and isovaleraldehyde (8 mg, 0.09 mmol) were condensed to afford the title compound (**2.79**) (31 mg, 30%) as a pale yellow crystalline solid, m.p. 118-119 °C.

ν_{\max} (KBr, cm⁻¹); 3364, 3289, 2954, 2968, 1612, 1462, 1363, 1098; δ_H (CDCl₃); 0.88 (6H, br s, 2 × CH₃), 1.33 (6H, br s, 2 × CH₃), 1.42 (1H, m, CH), 1.68 (3H, s, CH₃), 1.75 (3H, s, CH₃), 1.77 (3H, s, CH₃), 1.82 (3H, s, CH₃), 2.14 (2H, m, CH), 2.66 (3H, s, CH₃), 2.69 (3H, s, CH₃), 3.30 (2H, d, *J* 6.3,

CH₂), 3.41 (2H, d, *J* 6.7, CH₂), 4.52 (3H, s, OCH), 4.73 (H, t, *J* 7.7), 5.19 (2H, *J* 6.6), 6.20 (1H, br s, OH), 9.58 (1H, br s, OH), 10.06 (1H, br s, OH), 15.62, (1H, br s, OH), 15.70(1H, br s, OH); δ_c (CDCl₃); 17.6 (CH₃), 17.9 (CH₃), 21.9 (CH₃), 22.0 (CH₃), 22.5 (CH₂), 22.6 (CH₃), 22.5 (CH₂), 23.2 (CH₂), 25.5 (CH₃), 25.6 (CH₃), 27.0 (CH), 28.8 (CH), 30.6 (CH₃), 32.6 (CH₃), 39.6 (CH₂), 70.2 (CH), 104.2 (C_q), 106.3 (C_q), 108.3 (C_q), 108.8 (C_q), 114.2 (C_q), 116.7 (C_q), 121.6 (CH), 123.2 (CH), 131.0 (C_q), 136.7 (C_q), 158.2 (C_q), 158.3 (C_q), 160.0 (C_q), 160.2 (C_q), 160.3 (C_q), 162.6 (C_q), 204.1 (C_q), 204.3 (C_q).

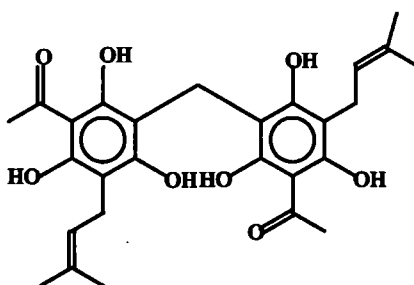
1-[(2,6-dihydroxy-4-(3-methyl-but-2-enyloxy)-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl)-1-(2,4,6-trihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl)]-3-methylbutane (2.80)



Following the general procedure the compound **2.76** (50 mg, 0.16 mmol), demethylacronylin (**2.6**) (38 mg, 0.16 mmol) and isovaleraldehyde (8 mg, 0.08 mmol) were condensed to afford the title compound (**2.80**) (30 mg, 30%) as a pale yellow crystalline solid, m.p. 99-100 °C.

ν_{\max} (KBr, cm^{-1}); 3393, 3246, 2968, 2954, 2915, 1611, 1461, 1320, 1282, 1098; δ_{H} (CDCl_3); 0.84 (6H, br s, $2 \times \text{CH}_3$), 1.24 (1H, m, CH), 1.68 (6H, s, $2 \times \text{CH}_3$), 1.73 (3H, s, CH_3), 1.75 (6H, s, $2 \times \text{CH}_3$), 1.79 (3H, s, CH_3), 2.14 (2H, m, CH_2), 2.62 (3H, s, CH_3), 2.66 (3H, s, CH_3), 3.33 (2H, d, J 6.6, CH_2), 3.40 (2H, d, J 6.6, CH_2), 4.50 (2H, s, J 6.8, CH_2), 4.74 (1H, t, J 7.7, CH), 5.23 (2H, J 6.6, $2 \times \text{CH}$), 5.40 (1H, t, J 6.8, CH), 6.20 (1H, br s, OH), 9.58 (1H, br s, OH), 10.06 (1H, br s, OH), 15.62 (1H, br s, OH), 15.70 (1H, br s, OH); δ_{C} (CDCl_3); 18.0 (CH_3), 18.2 (CH_3), 18.4 (CH_3), 22.3 (CH_2), 22.7 (CH_3), 22.8 (CH_3), 23.1 (CH_2), 25.5 (CH_3), 25.6 (CH_3), 26.0 (CH_3), 27.0 (CH), 28.8 (CH), 32.4 (CH_3), 32.6 (CH_3), 39.6 (CH_4), 65.2 (CH_2), 104.9 (C_q), 106.3 (C_q), 108.4 (C_q), 108.9 (C_q), 113.2 (C_q), 116.8 (C_q), 118.9 (CH), 121.5 (CH), 123.2 (CH), 131.8 (C_q), 136.7 (C_q), 139.1 (C_q), 158.2 (C_q), 158.6 (C_q), 160.3 (C_q), 161.0 (C_q), 162.0 (C_q), 165.9 (C_q), 204.2 (C_q), 204.3 (C_q).

Synthesis of *bis*-2,4,6-trihydroxy-3-isopentenyl acetophenone (2.81)

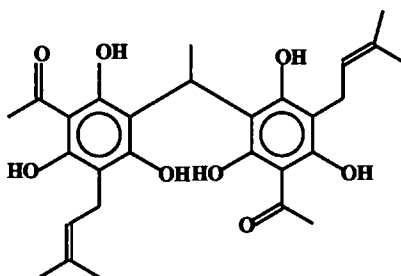


To a solution of demethylacronylin (2.6) (100 mg, 0.42 mmol) in a mixture of dichloro methane:diethyl ether (4:1, v/v) was added dropwise a solution of formaldehyde (18 mg, 0.21 mmol) and Amberlyst 15 (100 mg). The reaction mixture was gently refluxed for 2 h. The Amberlyst was filtered and filtrate

was concentrated *in vacuo*. The crude product was purified by dry column chromatography to obtain the title compound (**2.81**) (46 mg, 45%) as a yellow crystalline solid, m.p. 130-131 °C.

ν_{\max} (KBr, cm^{-1}); 3335, 1634, 1610, 1598, 1282, 1072; δ_{H} (CDCl_3); 1.76 (6H, s, $2 \times \text{CH}_3$), 1.82 (6H, s, $2 \times \text{CH}_3$), 2.62 (6H, s, $2 \times \text{CH}_3$), 3.47 (4H, d, J 6.3, $2 \times \text{CH}_2$), 3.78 (2H, s, CH_2), 5.18 (2H, t, J 6.3, CH).

Synthesis of 1,1 di-(2,4,6-trihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl)-ethane (2.82**)**

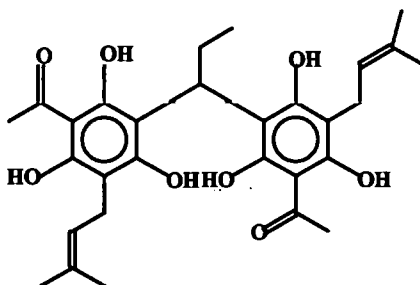


To a solution of demethylacronylin (**2.6**) (100 mg, 0.42 mmol) in a mixture of dichloro methane:diethyl ether (4:1, v/v) was added dropwise acetaldehyde (10 mg, 21 mmol) and Amberlyst 15 (0.1 g). The reaction mixture was stirred at room temperature for 12 h. Amberlyst was filtered and filtrate was concentrated *in vacuo*. The crude product was dry column chromatographed to obtain the title compound (**2.82**) (47 mg, 45%) as a yellow crystalline solid, m.p. 128-129 °C.

ν_{\max} (KBr, cm^{-1}) ; 3335, 1634, 1606, 1560, 1369; δ_{H} (CDCl_3); 1.76 (3H, br s, CH_3), 1.78 (3H, s, CH_3), 1.84 (3H, s, CH_3), 2.69 (6H, s, $2 \times \text{CH}_3$), 3.39 (4H, d,

J 6.8, CH₂), 4.86 (1H, br s, CH), 5.20 (2H, d, J 6.8, CH); δ_C (CDCl₃); 17.9 (2 \times CH₃), 22.2 (2 \times CH₂), 24.6 (CH₃), 25.8 (2 \times CH₃), 104.6 (2 \times C_q), 108.2 (2 \times C_q), 113.3 (2 \times C_q), 158.2 (2 \times C_q), 160.0 (2 \times C_q), 160.2 (2 \times C_q), 204.2 (2 \times C_q).

Synthesis of 1,1 di-[2,4,6-trihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl]-propane (2.83)



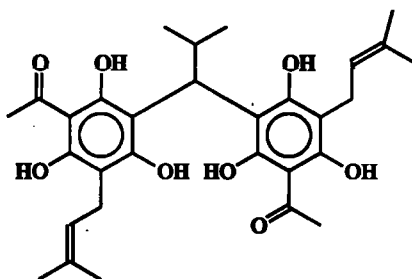
To a solution of demethylacronylin (2.6) (100 mg, 0.42 mmol) in a mixture of dichloro methane:diethyl ether (4:1, v/v) was added dropwise propanaldehyde (12 g, 0.21 mmol) and Amberlyst 15 (100 mg). The reaction mixture was stirred at room temperature for 12 h. Amberlyst was filtered and filtrate was concentrated *in vacuo*. The crude product was purified by dry column chromatography to obtain the title compound (2.83) (48 mg, 45%) as a yellow crystalline solid, m.p. 124-125 °C.

ν_{\max} (KBr, cm⁻¹); 3289, 1625, 1590, 1413, 1363, 1233; δ_H (CDCl₃); 0.87 (3H, t, J 7.1, CH₃), 1.46 (2H, m, CH₂), 1.68 (3H, br s, CH₃), 1.71 (3H, s, CH₃), 1.78 (3H, s, CH₃), 1.84 (3H, s, CH₃), 2.69 (6H, s, 2 \times CH₃), 3.39 (4H, d, J 6.8, CH₂), 4.69 (1H, br s, CH), 5.20 (2H, d, J 6.8, CH); δ_C (CDCl₃); 17.9 (2 \times

CH₃), 22.2 (2 × CH₂), 24.6 (CH₃), 25.8 (2 × CH₃), 105.1 (2 × C_q), 106.0 (C_q), 106.1 (C_q), 109.4 (2 × C_q), 122.2 (CH), 122.7 (CH), 134.5 (C_q), 136.9 (C_q), 158.2 (2 × C_q), 160.2 (C_q), 160.5 (2 × C_q), 161.2 (C_q), 205.0 (2 × C_q).

(2 × C_q),

Synthesis of 1,1 di-[2,4,6-trihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl]-propane-2-methyl-propane (2.84)

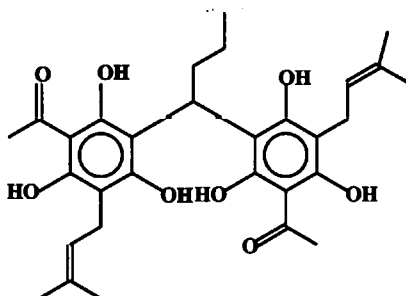


To a solution of demethylacronylin (2.6) (100 mg, 0.42 mmol) in a mixture of dichloro methane:diethyl ether (4:1, v/v) was added dropwise 2-methylpropanal (12 g, 0.21 mmol) and Amberlyst 15 (0.1 g) and stirred at room temperature for 12 h. Amberlyst was filtered and filtrate was concentrated *in vacuo*. The crude product was purified by dry column chromatography to obtain the title compound (2.84) (50 mg, 45%) as a yellow crystalline solid, m.p. 132-133 °C.

ν_{\max} (KBr, cm⁻¹); 3334, 1634, 1598, 1448, 1282, 1150; δ_{H} (CD₃COCD₃); 0.86 (6H, br s, 2 × CH₃), 1.67 (6H, s, 2 × CH₃), 1.84 (6H, s, 2 × CH₃), 2.65 (6H, s, 2 × CH₃), 3.10 (1H, m, CH), 3.46 (4H, d, *J* 7, 2 × CH₂), 4.27 (1H, d, *J* 11.2, CH), 5.27 (2H, t, *J* 7, 2 × CH), 9.20 (1H, s, OH), 13.21 (1H, s, OH); δ_{C}

(CD₃COCD₃); 17.0 (CH₃), 17.6 (CH₃), 18.0 (CH₃), 18.6 (CH₃), 22.1 (2 × CH₃), 28.3 (CH), 32.5 (CH₃), 32.6 (CH₃), 39.2 (CH), 105.0 (2 × C_q), 106.1 (C_q), 106.5 (C_q), 109.5 (2 × C_q), 121.4 (CH), 122.7 (CH), 136.5 (C_q), 136.9 (C_q), 158.0 (2 × C_q), 160.2 (C_q), 161.0 (2 × C_q), 161.2 (C_q), 204.0 (2 × C_q).

Synthesis of 1,1 di-[2,4,6-trihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)-phenyl] -butane (2.85)

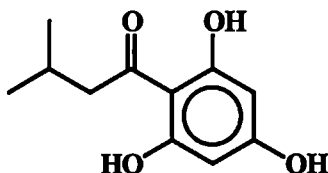


To a solution of demethylacronylin (2.6) (100 mg, 0.42 mmol) in a mixture of dichloro methane:diethyl ether (4:1, v/v) was added dropwise butanal (12 g, 0.21 mmol) and Amberlyst 15 (0.1 g) and gently refluxed for 2 h. The Amberlyst residue was filtered and filtrate was concentrated *in vacuo*. The crude product was purified by dry column chromatography to obtain the title compound (2.85) (50 mg, 45%) as a white crystalline solid, m.p. 140-141 °C.

ν_{max} (KBr, cm⁻¹); 3289, 1625, 1608, 1413, 1360, 1220; δ_{H} (CDCl₃); 0.92 (3H, dd, *J* 14.0, 6.5, CH₃), 1.73 (2H, m, CH₂), 1.77 (6H, s, 2 × CH₃), 1.86 (6H, s, 2 × CH₃), 2.12 (CH₂), 2.67 (6H, s, 2 × CH₃), 3.40 (4H, d, *J* 7.1, 2 × CH₂), 4.71 (1H, t, *J* 7.8, CH), 5.20 (2H, t, *J* 7.0, 2 × CH); δ_{C} (CDCl₃); 10.5 (CH₃), 17.8 (CH₃), 17.9 (CH₃), 22.4 (2 × CH₃), 22.9 (CH₂), 25.8 (2 × CH₃), 28.3 (CH),

32.7 ($2 \times \text{CH}_3$), 38.5 (CH_2), 105.0 (C_q), 105.5 (C_q), 106.0 (C_q), 106.3 (C_q), 109.4 ($2 \times \text{C}_q$), 121.4 (CH), 122.7 (CH), 135.6 (C_q), 136.8 (C_q), 158.1 ($2 \times \text{C}_q$), 160.0 (C_q), 161.1 ($2 \times \text{C}_q$), 161.2 (C_q), 204.0 ($2 \times \text{C}_q$).

Synthesis of 2,4,6 trihydroxyisovalerophenone (2.91)

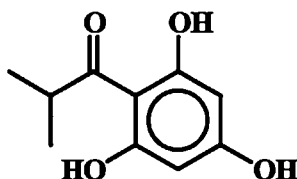


Isovaleryl chloride (5.7 g, 47 mmol) was added dropwise to a stirred solution of phloroglucinol (5.0 g, 39 mmol) and AlCl_3 (7.8 g, 58 mmol) in nitrobenzene at 0°C under N_2 . The solution was stirred for 48 h at room temperature. The reaction mixture was poured onto ice cold water (25 ml) and extracted with diethyl ether (3×100 ml). The combined organic extracts were extracted with 10% aqueous NaOH solution (3×100 ml). The aqueous extracts were neutralised with 2N HCl and extracted with diethyl ether (3×50 ml). The combined organic extracts were washed with water (3×50 ml), dried over MgSO_4 and evaporated *in vacuo*. The crude product was purified by dry column flash chromatography using a mixture of diethyl ether:petroleum ether (1:2, v/v) to afford the title compound **2.91** (7.5 g, 90%) as a yellow crystalline solid, m.p. $123\text{--}124^\circ\text{C}$.

ν_{max} (KBr, cm^{-1}); 3320, 3013, 2980, 1710, 1622, 1605, 1520, 1443;
 δ_{H} (CDCl_3); 0.94 (6H, d, J 6.7, $2 \times \text{CH}_3$), 2.24 (1H, sept, J 6.7, CH), 2.96 (2H,

d, J 6.7, CH₂), 5.94 (2H, s, 2 × Ar-H), 9.31 (1H, s, OH), 13.2 (1H, s, OH); δ_{C} (CDCl₃); 23.0 (CH₃), 25.3 (CH), 25.8 (CH₃), 53.1 (CH₂), 95.8 (2 × CH), 105.3 (C_q), 165.1 (2 × C_q), 165.4 (C_q), 206.0 (C_q).

Synthesis of 2,4,6-trihydroxyisobutyrophenone (2.92)

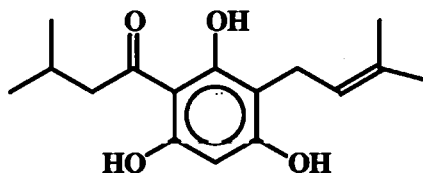


Isobutyryl chloride (4.2 g, 47 mmol) was added dropwise to a stirred solution of phloroglucinol (5.0 g, 39 mmol) and AlCl₃ (7.8 g, 58 mmol) in nitrobenzene at 0 °C under N₂. After 15 min, the solution was heated to reflux for 2 h. On cooling reaction mixture was poured onto ice cold water (25 ml) and extracted with diethyl ether (3 × 100 ml). The combined organic extracts were extracted with 10% aqueous NaOH solution (3 × 100 ml). The aqueous extracts were neutralised with 2N HCl and extracted with diethyl ether (3 × 50 ml). The combined organic extracts were washed with water (3 × 50 ml), dried over MgSO₄ and evaporated *in vacuo*. The crude product was purified by dry column flash chromatography using a mixture of diethyl ether:petroleum ether (1:1, v/v) to afford the title compound **2.92** (4.5 g, 60%) as an off white crystalline solid, m.p. 139-140 °C.

ν_{max} (KBr, cm⁻¹); 3324, 3030, 2980, 1710, 1630, 1605, 1527, 1453; δ_{H} (CD₃COCD₃) 1.17 (6H, d, J 6.7, 2 × CH₃), 4.00 (1H, sept, J 6.7, CH), 5.99

(2H, s, 2 × Ar-H), 9.25 (1H, s, OH), 13.41 (1H, s, OH); $\delta_{\text{C}}(\text{CD}_3\text{COCD}_3)$; 19.6 (2 × CH₃), 39.4 (CH), 96.0 (2 × CH), 104.2 (C_q), 165.0 (C_q), 165.2 (2 × C_q), 208.2 (C_q).

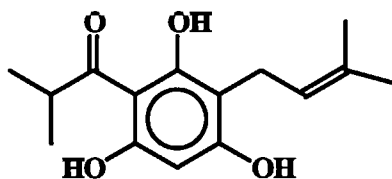
Synthesis of 3-methyl-1-[2,4,6-trihydroxy-3-(3-methyl-but-2-enyl)-phenyl]-butan-1-one (2.93)



Compound **2.91** (5.0 g, 23 mmol), was prenylated (prenyl bromide, 3.5g, 23 mmol) following the procedure similar to compound **2.6** (method i, page 177). to yield the compound **2.93** (1.6 g, 25%), as yellow solid, m.p. 138-139 °C

ν_{max} (KBr, cm⁻¹); 3425, 3135, 2070, 1715, 1605, 1517, 1450; $\delta_{\text{H}}(\text{CDCl}_3)$ 0.94 (6H, d, *J* 6.7, 2 × CH₃), 1.62 (3H, s, CH₃), 1.78 (3H, s, CH₃), 2.24 (1H, m, CH), 2.94 (2H, d, *J* 6.7, CH₂), 3.25 (2H, d, *J*, 7, CH₂), 5.21 (1H, t, *J*, 7, CH), 5.99 (1H, s, CH), 9.03 (1H, s, OH), 13.40 (1H, s, OH); $\delta_{\text{C}}(\text{CDCl}_3)$ 17.8 (CH₃), 22.3 (CH), 23.1 (CH₃), 25.2 (CH₃), 25.3 (CH), 25.8 (CH₃), 52.8 (CH₂), 98.2 (CH), 108.2 (C_q), 116.0 (C_q), 122.1 (CH), 136.2 (C_q), 160.4 (C_q), 165.1 (C_q), 165.2 (C_q), 205.9 (C_q).

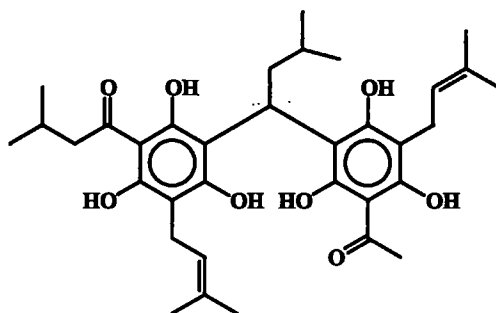
Synthesis of 2-methyl-1-[2,4,6-trihydroxy-3-(3-methyl-but-2-enyl)-phenyl]-propan-1-one (2.94)



Compound **2.92** (4.0 g, 20 mmol), was prenylated (prenyl bromide 3.0 g, 20 mmol) following the procedure similar to compound **2.6** (method i, page 177). to yield the compound **2.94** (1.0 g, 20%), as a light yellow solid, m.p. 165-166 °C.

ν_{\max} (KBr, cm^{-1}); 3325, 3035, 2970, 1710, 1610, 1605, 1517, 1450; δ_{H} (CDCl_3) 1.16 (6H, d, J 6.7, $2 \times \text{CH}_3$), 1.66 (3H, s, CH_3), 1.75 (3H, s, CH_3), 3.28 (2H, d, J , 6.8, CH_2), 4.00 (1H, sept, J 6.7, CH), 5.36 (1H, t, J 6.8, CH), 6.01 (H, s, Ar-H), 9.20 (1H, s, OH), 13.35 (1H, s, OH); δ_{C} (CD_3Cl_3); 17.6 (CH_3), 19.6 ($2 \times \text{CH}_3$), 21.6 (CH_2), 25.6 (CH_3), 39.4 (CH), 96.8 (CH), 106.2 (C_q), 116.7 (C_q), 122.4 (CH), 135.0 (C_q), 160.5 (C_q), 160.6 ($2 \times \text{C}_q$), 208.2 (C_q).

Synthesis of 1-[2,4,6-trihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)phenyl]-1-(2,4,6-trihydroxy-3-(3-methyl-1-oxobutyl)-5-(3-methyl-but-2-enyl)phenyl]-3-methylbutane (2.95)

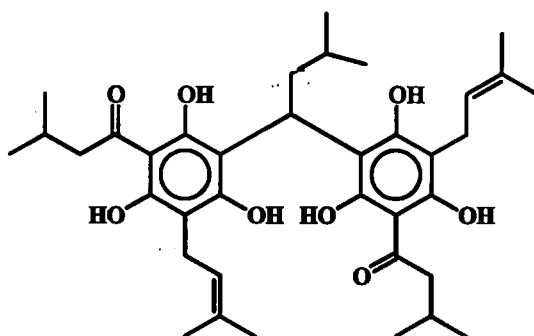


Demethylacronylin (**2.6**) (100 mg, 0.4 mmol) and compound **2.91** (117 mg, 0.4 mmol) were condensed with isovaleraldehyde (18 mg, 0.2 mmol) (followed the procedure similar to compound **2.10** in page 179) to obtain compounds **2.95** (36 mg, 30%), and **2.96** (25 mg, 20%) as yellow gummy semisolids.

ν_{\max} (KBr, cm^{-1}); 3330, 3023, 2990, 1720, 1612, 1605, 1525, 1443;
 δ_{H} (CDCl_3); 0.89 (6H, br s, $2 \times \text{CH}_3$), 0.97 (3H, d, J 6.8, CH_3), 0.99 (3H, d, J 6.8, CH_3), 1.25 (1H, m, CH), 1.74 (3H, s, CH_3), 1.76 (3H, s, CH_3), 1.80 (3H, s, CH_3), 1.81 (3H, s, CH_3), 2.21 (2H, m, CH_2), 2.24 (1H, d, J 6.8, CH), 2.62 (3H, s, CH_3), 2.98 (2H, d, J 6.3, CH), 3.24 (2H, d, J 7.0, CH_2), 3.39 (2H, d, J 7.0, CH_2), 4.81 (1H, br s, CH), 5.22 (1H, d, J 7.0, CH), 5.23 (1H, d, J 7.1, CH), 9.02 (1H, s, OH), 9.42 (1H, s, OH), 12.41 (1H, s, OH), 14.09 (1H, s, OH). δ_{C} (CDCl_3); 17.9 (CH_3), 18.1 (CH_3), 21.9 (CH_2), 22.3 (CH_2), 22.6 (CH_3), 22.7 ($2 \times \text{CH}_3$), 22.8 (CH_3), 25.3 (CH), 25.6 (CH_3), 26.1 (CH_3), 27.0 (CH),

29.0 (CH), 31.6 (CH₃), 39.8 (CH₂), 58.2 (CH₂), 104.9 (C_q), 105.3 (C_q), 106.8 (C_q), 108.2 (C_q), 108.3 (C_q), 116.7 (C_q), 121.6 (CH), 123.2 (CH), 131.6 (C_q), 136.7 (C_q), (C_q), 158.2 (C_q), 160.0 (C_q), 160.2 (C_q), 160.8 (C_q), 162.6 (C_q), 165.4 (C_q), 204.3 (C_q), 206.0 (C_q).

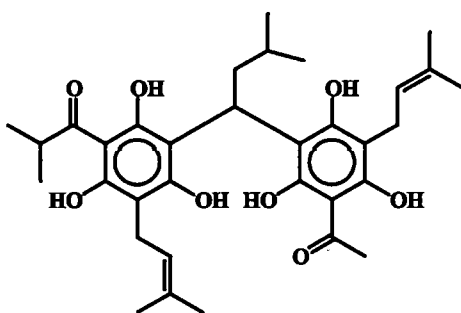
1,1 di-(2,4,6-trihydroxy-3-(3-methyl-1-oxobutyl)-5-(3-methyl-but-2-enyl)phenyl]-3-methylbutane (2.96)



ν_{\max} (KBr, cm⁻¹); 3420, 3110, 2970, 1720, 1608, 1605, 1515, 1440;
 δ_{H} (CDCl₃); 0.86 (6H, br s, 2 × CH₃), 0.94 (12H, d, *J* 6.7, 4 × CH₃), 1.24 (1H, m, CH), 1.68 (3H, s, CH₃), 1.75 (3H, s, CH₃), 1.76 (3H, s, CH₃), 1.81 (3H, s, CH₃), 2.14 (1H, m, CH₂), 2.24 (2H, m, 2 × CH), 2.96 (4H, m, 2 × CH₂), 3.25 (2H, d, *J* 6.8, CH₂), 3.29 (2H, d, *J* 7.0, CH₂), 4.81 (1H, m, CH), 5.22 (1H, d, *J* 7.0, CH), 5.23 (1H, d, *J* 6.8, CH), 9.01 (1H, s, OH), 10.02 (1H, s, OH), 13.24 (1H, s, OH); δ_{C} (CDCl₃); 17.9 (CH₃), 18.1 (CH₃), 22.3 (CH₂), 22.6 (CH₃), 22.8 (CH₃), 23.0 (2 × CH₃), 23.1 (CH₂), 25.3 (2 × CH), 25.6 (CH₃), 25.7 (CH₃), 25.8 (2 × CH₃), 27.0 (CH), 30.0 (CH), 39.4 (CH₂), 104.8 (2 × C_q), 106.8 (2 ×

C_q), 108.4 ($2 \times C_q$), 121.6 (CH_2), 123.2 (CH_2), 131.6 (C_q), 136.7 (C_q), 158.3 ($2 \times C_q$), 160.0 ($2 \times C_q$), 206.8 ($2 \times C_q$).

Synthesis of 1-[2,4,6-trihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)phenyl]-1-(2,4,6-trihydroxy-3-(2-methyl-1-oxopropyl)-5-(3-methyl-but-2-enyl)phenyl]-3-methylbutane (2.97)

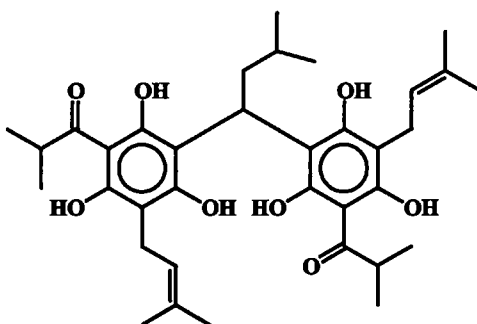


Demethylacronylin (2.6) (100 mg, 0.4 mmol) and compound 2.94 (111 mg, 0.4 mmol) were condensed with isovaleraldehyde (18 mg, 0.2 mmol) (followed the procedure similar to compound 2.10 in page 179) to obtain compounds 2.97 (15 mg, 17%), and 2.98 (20 mg, 20%) as yellow gummy solids.

ν_{\max} (KBr, cm^{-1}); 3428, 3145, 2060, 1725, 1608, 1517, 1450; $\delta_H(CDCl_3)$ 0.84 (6H, br s, $2 \times CH_3$), 1.14 (6H, d, J 7, $4 \times CH_3$), 1.24 (1H, m, CH), 1.73 (3H, s, CH_3), 1.75 (3H, s, CH_3), 1.80 (3H, s, CH_3), 1.82 (3H, s, CH_3), 2.15 (1H, m, CH_2), 3.31 (2H, d, J 7, CH_2), 3.32 (2H, d, J 7.0, CH_2), 3.74 (1H, sept, J 7.1, CH), 4.76 (1H, m, CH), 5.21 (1H, d, J 7.0, CH), 5.20 (1H, d, J 7, CH), 9.01 (1H, s, OH), 10.02 (1H, s, OH), 13.24 (1H, s, OH); $\delta_C(CDCl_3)$ 17.9 (CH_3),

18.1 (CH₃), 19.9 (2 × CH₃), 21.9 (CH₂), 22.3 (CH₂), 22.6 (CH₃), 22.8 (CH₃), 25.6 (CH₃), 26.1 (CH₃), 27.0 (CH), 28.6 (CH), 31.6 (CH₃), 39.4 (CH₂), 39.6 (CH), 104.8 (C_q), 106.2 (C_q), 108.6 (C_q), 108.8 (C_q), 113.2 (C_q), 116.7 (C_q), 121.6 (CH), 123.4 (CH), 135.1 (C_q), 136.7 (C_q), 158.3 (C_q), 158.4 (C_q), 160.3 (C_q), 160.8 (C_q), 160.9 (C_q), 162.6 (C_q), 204.3 (C_q), 206.0 (C_q).

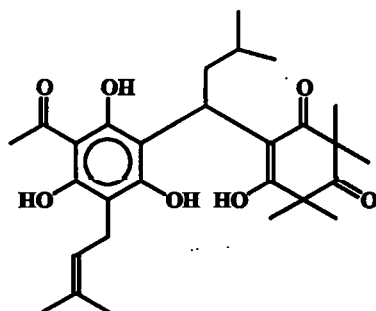
1,1di-[(2,4,6-trihydroxy-3-(2-methyl-1-oxopropyl)-5-(3-methyl-but-2-enyl)phenyl]-3-methylbutane (2.98)



ν_{\max} (KBr, cm⁻¹); 3420, 3135, 2060, 1735, 1609, 1515, 1450; δ_{H} (CDCl₃) 0.84 (6H, br s, 2 × CH₃), 1.15 (12H, d, *J* 7, 4 × CH₃), 1.24 (1H, m, CH), 1.73 (3H, s, CH₃), 1.75 (3H, s, CH₃), 1.81 (3H, s, CH₃), 1.82 (3H, s, CH₃), 2.14 (1H, m, CH₂), 3.30 (2H, d, *J* 7, CH₂), 3.32 (2H, d, *J* 7.0, CH₂), 3.70 (1H, sept, *J* 7.0, CH), 4.74 (1H, m, CH), 5.20 (1H, d, *J* 7.0, CH), 5.22 (1H, d, *J* 7, CH), 9.10 (1H, s, OH), 10.13 (1H, s, OH), 14.24 (1H, s, OH); δ_{C} (CDCl₃) 18.0 (CH₃), 18.1 (CH₃), 20.1 (2 × CH₃), 21.9 (CH₂), 22.5 (CH₂), 22.6 (2 × CH₃), 25.6 (CH₃), 26.1 (CH₃), 27.0 (CH), 28.8 (CH), 39.4 (CH₂), 39.6 (2 × CH), 106.3 (2

$\times C_q$), 108.6 ($2 \times C_q$), 116.7 ($2 \times C_q$), 121.4 (CH), 123.2 (CH), 131.1 (C_q), 136.7 (C_q), 160.3 ($2 \times C_q$), 160.9 ($2 \times C_q$), 162.6 ($2 \times C_q$), 204.6 ($2 \times C_q$).

Synthesis of 1-[2,4,6-trihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)phenyl]-1-(3,3,5,5-tetramethylcyclohexane-2,4-dione)]-3-methylbutane (2.103)

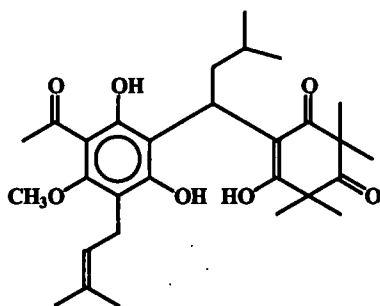


To a stirred solution of pyrrolidine adduct (2.110) (50 mg, 0.15 mmol), demethylacronylin (2.6) (36 mg, 0.15 mmol) in dry THF at 0 °C and pTSA (30 mg, 0.15 mmol) were added. After warming to the room temperature over 1 h, THF was removed *in vacuo*, added water (15 ml) and the product extracted with diethyl ether (3×20 ml). The combined extracts were washed with water (10 ml), dried over $MgSO_4$ and the solvent evaporated *in vacuo*. The crude product was purified by preparative thin layer chromatography using a mixture of diethyl ether:petroleum ether (1:1, v/v) to afford the title compound (2.103) (35 mg, 46%) as a yellow solid, m.p. 120-121 °C.

ν_{max} (KBr, cm^{-1}); 3344, 3025, 2983, 2936, 2870, 1710, 1608, 1561, 1470; δ_H ($CDCl_3$); 0.84 (3H, d, J 4.0, CH_3), 0.86 (3H, d, J 4.0, CH_3), 1.23 (H, m, CH), 1.34 (6H, s, $2 \times CH_3$), 1.38 (6H, s, $2 \times CH_3$), 1.75 (3H, s, CH_3), 1.85 (3H, s,

CH₃), 2.04 (2H, m, CH₂), 2.68 (3H, s, CH₃), 3.32 (2H, d, *J* 7.0, CH₂), 5.25 (1H, d, *J* 7.0, CH), 14.01 (1H, s, OH); δ_C (CDCl₃); 17.6 (CH₃), 22.0 (CH₂), 22.6 (CH₃), 22.8 (CH₃), 24.5 (2 × CH₃), 24.8 (2 × CH₃), 25.3 (CH₃), 27.0 (CH), 31.2 (CH), 32.2 (CH₃), 39.3 (CH₂), 51.4 (C_q), 51.7 (C_q), 106.2 (C_q), 108.2 (C_q), 116.2 (C_q), 124.5 (CH), 131.2 (C_q), 160.0 (C_q), 160.6 (C_q), 162.2 (C_q), 189.0 (C_q), 189.2 (C_q), 203.2 (C_q), 213.2 (C_q).

Synthesis of 1-[2,6-dihydroxy-4-methoxy-3-oxoethyl-5-(3-methyl-but-2-enyl)phenyl]-1-(3,3,5,5-tetramethylcyclohexane-2,4-dione)]-3-methylbutane (2.104)

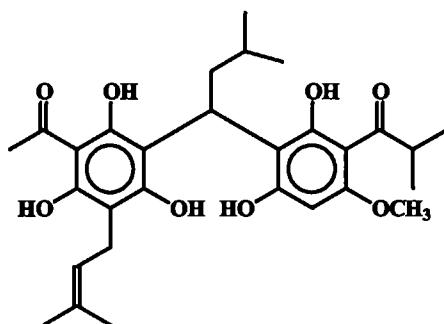


To a stirred solution of pyrrolidine adduct (2.110) (50 mg, 0.15 mmol), acronylin (2.5) (36 mg, 0.15 mmol) in dry THF at 0 °C and p-TSA (30 mg, 0.15 mmol) were added. After warming to the room temperature over 1 h, THF was removed *in vacuo*, added water (15 ml) and the product extracted with diethyl ether (3 × 20 ml). The combined extracts were washed with water (10 ml), dried over MgSO₄ and the solvent evaporated *in vacuo*. The crude product was purified by preparative thin layer chromatography using a mixture

of diethyl ether:petroleum ether (1:1, v/v) to afford the title compound (2.104) (35 mg, 46%) as a yellow solid, m.p. 124-125 °C.

ν_{\max} (KBr, cm^{-1}); 3360, 3010, 2980, 2936, 2870, 1700, 1608, 1555, 1470; δ_{H} (CDCl_3); 0.83 (3H, d, J 3.8, CH_3), 0.84 (3H, d, J 3.8, CH_3), 1.26 (2H, m, CH_2), 1.37 (6H, s, $2 \times \text{CH}_3$), 1.38 (6H, s, $2 \times \text{CH}_3$), 1.70 (1H, m, CH), 1.75 (3H, s, CH_3), 1.75 (3H, s, CH_3), 1.84 (3H, s, CH_3), 2.68 (3H, s, CH_3), 3.21 (2H, d, J 7.0, CH_2), 3.61 (3H, s, CH_3), 4.40 (1H, br s, CH), (5.20 (1H, d, J 7.0, CH), 13.96 (1H, s, OH); δ_{C} (CDCl_3); 17.8 (CH_3), 21.8 (CH_2), 22.6 ($2 \times \text{CH}_3$), 24.5 ($2 \times \text{CH}_3$), 24.6 ($2 \times \text{CH}_3$), 25.8 ($2 \times \text{CH}_3$), 27.0 ($2 \times \text{CH}_3$), 28.6 (CH), 32.8 (CH_3), 39.3 (CH_2), 51.7 (C_q), 53.0 (C_q), 60.0 (CH_3), 106.3 (C_q), 106.4 (C_q), 108.6 (C_q), 116.7 (C_q), 124.1 (CH), 160.6 (C_q), 160.9 (C_q), 162.6 (C_q), 188.2 (C_q), 188.6 (C_q), 203.7 (C_q), 214.0 (C_q).

**Synthesis of 1-[2,6-dihydroxy-3-(2-methyl-1-oxopropyl)-4-methoxyphenyl
)-1-(2,4,6-trihydroxy-3-oxoethyl-5-(3-methyl-but-2-enyl)phenyl]-3-
methylbutane (2.105)**

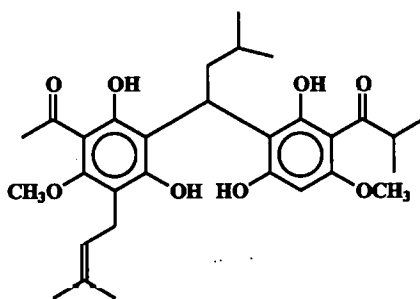


To a stirring solution of pyrrolidine adduct (2.112) (50 mg, 0.15 mmol), demethylacronylin (2.6) (36 mg, 0.15 mmol) in dry THF at 0 °C and p-TSA (30 mg, 0.15 mmol) were added. After warming to the room temperature over 1 h, THF was removed *in vacuo*, added water (15 ml) and the product extracted with diethyl ether (3 × 20 ml). The combined extracts were washed with water (10 ml), dried over MgSO₄ and the solvent evaporated *in vacuo*. The crude product was purified by preparative thin layer chromatography using a mixture of diethyl ether:petroleum ether (1:1, v/v) to afford the title compound (2.105) (35 mg, 46%) as a yellow solid, m.p. 115-116 °C.

ν_{\max} (KBr, cm⁻¹); 3586, 3410, 2973, 2938, 1660, 1601, 1025; δ_{H} (CDCl₃); 0.79 (3H, s, CH₃), 0.80 (3H, s, CH₃), 1.13 (3H, s, CH₃), 1.15 (3H, s, CH₃), 1.25 (2H, m, CH₂), 1.63 (3H, s, CH₃), 1.72 (1H, m, CH), 1.74 (3H, s, CH₃), 2.61 (3H, s, CH₃), 3.25 (2H, d, *J* 7.0, CH₂), 3.75 (1H, m, CH), 3.85 (3H, s, CH₃), 4.48 (1H, t, *J* 7.0, CH), 5.12 (1H, t, *J* 7.0, CH), 5.92 (1H, s, ArH), 9.0

(1H, s, OH), 10.02 (1H, s, OH), 14.02 (1H, s, OH), 16.21 (1H, s, OH); δ_C (CDCl₃); 16.8 (CH₃), 19.4 (2 × CH₃), 21.9 (CH₂), 22.6 (2 × CH₃), 25.8 (CH₃), 27.4 (CH), 28.6 (CH), 32.7 (CH₃), 39.6 (CH₂), 39.7 (CH), 55.7 (CH₃), 96.8 (CH), 105.3 (C_q), 105.6 (C_q), 106.2 (C_q), 107.7 (C_q), 116.2 (C_q), 124.1 (CH), 130.7 (C_q), 160.7 (C_q), 162.3 (C_q), 162.8 (C_q), 163.2 (C_q), 164.9 (C_q), 167.5 (C_q), 203.7 (C_q), 210.5 (C_q).

Synthesis of 1-[2,6-dihydroxy-3-(2-methyl-1-oxopropyl)-4-methoxyphenyl]-1-(2,6-dihydroxy-3-oxoethyl-4-methoxy-5-(3-methyl-but-2-enyl)phenyl)-3-methylbutane (2.106)

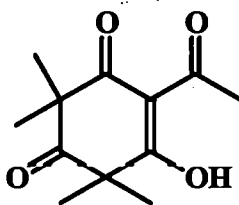


To a stirring solution of pyrrolidine adduct (2.113) (50 mg, 0.15 mmol), demethylacronylin (2.6) (36 mg, 0.15 mmol) in dry THF at 0 °C and pTSA (30 mg, 0.15 mmol) were added. After warming to room temperature over 1 h, THF was removed *in vacuo*, added water (15 ml) and the product was extracted with diethyl ether (3 × 20 ml). The combined extracts were washed with water (10 ml), dried over MgSO₄ and the solvent evaporated *in vacuo*. The crude product was purified by preparative thin layer chromatography

using a mixture of diethyl ether:petroleum ether (1:1, v/v) to afford the title compound (**2.106**) (35 mg, 46%) as a yellow solid, 110-112 °C.

ν_{\max} (KBr, cm^{-1}); 3550, 3412, 2973, 2990, 1630, 1605, 1467, 1440, 1026; δ_{H} (CDCl_3); 0.83 (3H, s, CH_3), 0.84 (3H, s, CH_3), 1.13 (3H, s, CH_3), 1.15 (3H, s, CH_3), 1.70 (1H, m, CH), 1.76 (3H, s, CH_3), 1.81 (3H, s, CH_3), 2.21 (2H, m, CH_2), 2.62 (3H, s, CH_3), 3.39 (2H, d, J 7.0, CH_2), 3.61 (3H, s, CH_3), 3.75 (1H, m, CH), 3.86 (3H, s, CH_3), 4.81 (1H, t, J 7.0, CH), 5.23 (1H, t, J 7.0, CH), 5.91 (1H, s, ArH), 9.10 (1H, s, OH), 10.21 (1H, s, OH), 12.5 (1H, s, OH), 13.2 (1H, s, OH); δ_{C} (CDCl_3); 17.9 (CH_3), 19.3 (CH_3), 19.4 (CH_3), 22.3 (CH_2), 22.6 (CH_3), 22.8 (CH_3), 25.6 (CH_3), 27.0 (CH), 29.0 (CH), 31.6 (CH_3), 39.6 (CH), 39.8 (CH_2), 55.7 (CH_3), 60.0 (CH_3), 96.8 (CH), 105.2 (C_q), 106.1 (C_q), 106.8 (C_q), 108.3 (C_q), 116.7 (C_q), 121.6 (CH), 131.6 (C_q), 160.2 (C_q), 160.8 (C_q), 162.6 (C_q), 163.2 (C_q), 167.5 (C_q), 204.3 (C_q), 209.2 (C_q).

Synthesis of 1-hydroxy-2-oxoethyl-4,4,6,6-tetramethylcyclohexane-3,5-dione (2.107**)**

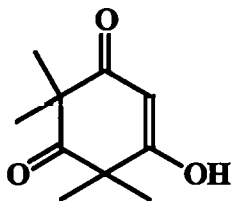


Sodium (10 g, 0.43 mol) was added slowly to methanol (100 ml) with cooling until the formation of NaOMe was completed. 2,4,6-Trihydroxyacetophenone (**2.7**) (10.0 g, 54 mmol) and iodomethane (34 ml, 54 mmol) were added

successively and the mixture was heated to reflux for 3 h. On cooling, solvent was removed *in vacuo* and added 2N HCl (100 ml). The resultant solid was filtered, dissolved in saturated NaHCO₃ solution, acidified with 10% HCl and filtered to afford the title compound (**2.107**) (12 g, 99%) as a pale yellow crystalline solid, m.p. 58-60 °C.

ν_{\max} (KBr, cm⁻¹); 3025, 2983, 2936, 2875, 1721, 1561, 1472; δ_{H} (CD₃OCD₃); 1.41 (6H, s, 2 × CH₃), 1.46 (6H, s, 2 × CH₃), 2.61 (3H, s, CH₃), 18.25 (1H, s, OH); δ_{C} (CD₃OCD₃); 24.1 (2 × CH₃), 24.6 (2 × CH₃), 27.3 (CH₃), 52.5 (C_q), 57.3 (C_q), 110.4 (C_q), 201.7 (C_q), 205.9 (C_q), 206.3 (C_q), 210.2 (C_q).

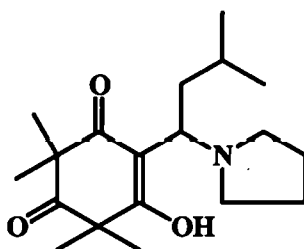
Synthesis of 1-hydroxy-4,4,6,6-tetramethylcyclohexene-3,5-dione (**2.108**)



The trione **2.107** (10 g, 44.6 mmol) was dissolved in 50% H₂SO₄ (100 ml) and the mixture was heated to reflux for 24 h. On cooling, water (200 ml) was added and the product removed by filtration. Recrystallisation of the crude product with diethyl ether afforded the title compound (**2.108**) (6.0 g, 75%) as a off white crystalline solid, m.p. 180-182 °C.

ν_{\max} (KBr, cm^{-1}); 3024, 2980, 1709, 1473, 1385; δ_{H} (CD_3OCD_3); 1.33 (12H, s, $4 \times \text{CH}_3$), 5.50 (1H, s, CH); δ_{C} (CD_3OCD_3); 24.9 ($4 \times \text{CH}_3$), 51.7 ($2 \times \text{C}_q$), 102.0 (C_q), 188.0 ($2 \times \text{C}_q$), 214.0 (C_q).

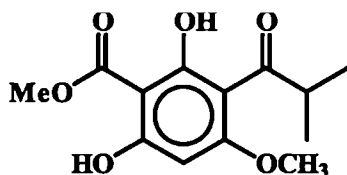
Synthesis of 1-(pyrrolidine)-1-(2-hydroxy-3,3,6,6-tetramethylcyclohexene-4,6-dione)-3-methylbutane (2.110)



To an ice cold solution of syncarpic acid (2.108) (2.0 g, 10.9 mmol) and pyrrolidine (0.84 g, 11 mmol) in dry diethyl ether (30 ml) was added isovaleraldehyde (1.0 g, 11 mmol) over 5 min. After stirring for 1 h, the resultant solid was filtered and washed with cold diethyl ether to afford the title compound (2.110) (2 g, 57%) as a white crystals, m.p. 52-54 °C.

ν_{\max} (KBr, cm^{-1}); 2981, 2930, 1702, 1586, 1522, 1466, 1405; δ_{H} (CDCl_3); 0.88 (3H, s, d, J 6.3, CH_3), 0.96 (3H, s, d, J 6, CH_3), 1.35 (6H, s, $2 \times \text{CH}_3$), 1.46 (1H, m, CH), 2.07 (2H, m, CH_2), 2.86 (2H, dd, J 9.6, 18.1, CH_2), 3.02 (2H, dd, J 8.3, 11.8, CH_2), 3.34 (2H, m, CH_2), 3.57 (2H, m, CH_2), 4.46 (1H, dd, J 3.5, 11.4, CH); δ_{C} (CDCl_3); 22.2 (CH_3), 22.8 (CH_3), 24.2 (CH_3), 24.6 (CH_3), 25.0 ($2 \times \text{CH}_3$), 25.1 ($2 \times \text{CH}_3$), 25.7 (CH_3), 40.3 (CH_2), 50.9 ($2 \times \text{C}_q$), 58.2 (CH_2), 52.9 (CH_2), 64.5 (CH), 102.4 (C_q), 192.1 ($2 \times \text{C}_q$), 216.9 (C_q).

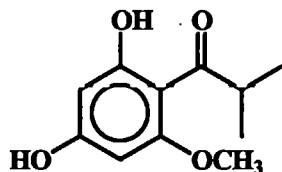
Synthesis of methyl 2,6-dihydroxy-4-methoxy-3-(2-methyl-1-oxopropyl)benzoate (2.111)



TiCl₄ (2.23 ml, 11 mmol) and isobutyl chloride (1.0 g, 10 mmol) were stirred at 0 °C under N₂. Methylbenzoate (**2.24**) (2.0 g, 10 mmol) in dry benzene (20 ml) was added dropwise and the mixture stirred at room temperature for another 20 min. A solution of 2N HCl (20 ml) was added, the organic layer was diluted with diethyl ether (25 ml), washed with saturated NaHCO₃, brine, water, then dried over MgSO₄ and concentrated *in vacuo* to obtain a gummy residue. The crude was purified by column chromatography to yield the title compound (**2.111**) (2.5 g, 83%) as a white crystalline solid, m.p. 91-93 °C.

ν_{\max} (KBr, cm⁻¹); 3436, 3023, 2979, 2873; 1653, 1595, 1449, 1343; δ_{H} (CDCl₃); 1.15 (6H, d, *J* 6.8, 2 × CH₃), 3.65 (1H, sept, *J* 6.8, CH), 3.91 (3H, s, OCH₃), 4.04 (3H, s, OCH₃), 6.00 (H, s, Ar), 12.54 (1H, s, OH), 15.45 (1H, s, OH); δ_{C} (CDCl₃); 19.1 (2 × CH₃), 39.9 (CH₃), 52.6 (CH₃), 55.9 (CH₃), 91.4 (CH₃), 95.9 (C_q), 104.7 (C_q), 165.9 (C_q), 169.4 (C_q), 171.6 (C_q), 183.5 (C_q), 210.3 (C_q).

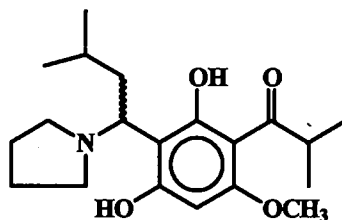
Synthesis of 4,6-dihydroxy-2-methoxyisobutyrophenone (2.112)



The compound (2.111) (2.0 g, 7.4 mmol) in 50% aqueous KOH (5 ml) and DMSO (10 ml) were heated to 115 °C for 2 h. On cooling, 10% HCl (20 ml) was added and the product extracted with diethyl ether (3 × 50 ml). The combined extracts were washed with water (3 × 50 ml), dried over MgSO₄ and concentrated *in vacuo*. The crude product was purified by column chromatography over silica gel using a mixture of diethyl ether:petroleum ether (2:3, v/v) to afford the title compound (2.112) (1.2 g, 80%) as a white crystalline solid, m.p.138-139 °C.

ν_{\max} (KBr, cm⁻¹); 3586, 2973, 2938, 2873, 1622, 1601, 1467, 1440; δ_{H} (CDCl₃); 1.16 (6H, d, *J* 6.4, 2 × CH₃), 3.75 (1H, sept, *J* 6.4, CH), 3.68 (3H, s, OCH₃), 5.85 (1H, s, OH), 5.93 (1H, d, *J* 2.0, Ar), 6.01 ((1H, d, *J* 2.0, Ar), 14.09 (1H, s, OH); δ_{C} (CDCl₃); 19.3 (2 × CH₃), 39.6 (CH), 55.7 (CH₃), 90.9 (CH), 96.7 (CH), 105.5 (C_q), 162.3 (C_q), 163.2 (C_q), 167.5 (C_q), 210.5 (C_q).

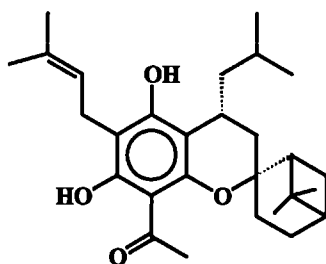
Synthesis of 1-(pyrrolidine)-1-(2,6-dihydroxy-4-methoxy-3-(2-methyl-1-oxopropyl))-3-methylbutane (2.113)



To an ice cold solution of (2.112) (1.0 g, 4.7 mmol) and isovaleraldehyde (0.40 g, 4.7 mmol) in dry diethyl ether (30 ml) was added pyrrolidine (0.33 g, 4.7 mmol) over 5 min. After stirring for 1 h, the resulting solid was filtered and washed with cold diethyl ether then dried over MgSO_4 to afford the title compound (2.113) (1.5 g, 93%) as a light brown solid.

ν_{max} (KBr, cm^{-1}): 2956, 2868, 1614, 1469, 1389; δ_{H} (CDCl_3): 0.81 (3H, d, J 6.7 CH₃), 0.99 (3H, d, J 6.7 CH₃), 1.15 (3H, d, J 6.8 CH₃), 1.16 (3H, d, J 6.7 CH₃), 1.42 (1H, d, J 6.7 CH), 1.60 (1H, m, CH₂), 1.77 (1H, m, CH₂), 1.84 (4H, m, $2 \times \text{NCH}_2\text{CH}_2$), 2.95 (4H, m, $2 \times \text{NCH}_2$), 3.76 (1H, sept, J 6.8 CH), 3.82 (3H, s, OCH₃), 4.03 (1H, dd, J 3.7, 10.7, CH), 5.84 (1H, s, Ar-H); δ_{C} (CDCl_3): 19.3 (CH₃), 19.6 (CH₃), 22.6 (CH₃), 23.4 ($2 \times \text{CH}_3$), 24.4 (CH₃), 25.1 (CH), 39.2 (CH), 42.8 (CH₂), 52.0 ($2 \times \text{C}_q$), 55.3 (OCH₃), 60.7 (CH), 92.0 (CH), 102.8 (C_q), 106.2 (C_q), 162.3 (C_q), 164.7 (C_q), 167.4 (C_q), 209.5 (C_q).

Synthesis of 5,7-dihydroxy-8-oxoethyl-6-isopentenyl-4-isobutyl-3',3'-dimethyl-2',4'-methanospiro-(chroman-2-,1-cyclohexane) (2.116)

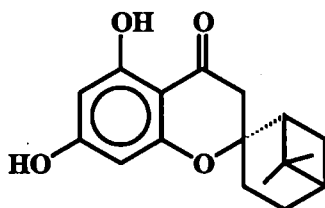


To a solution of **2.127** (700 mg, 1.8 mmol) in absolute dioxane (5 ml) $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (1 ml) was added slowly. The reaction mixture was warmed to 40°C and stirred for 30 min. A solution of 2-methyl-but-3-ene-2-ol (161 mg, 1.8 mmol) in dry dioxane (2 ml) was added to the mixture and stirred for another 2 h. The reaction mixture was cooled to room temperature, diluted with diethyl ether (20 ml), washed with water (3×20 ml), then dried over MgSO_4 and concentrated *in vacuo*. The resulting gum was purified by column chromatography on silica gel eluting with diethyl ether:petroleum ether (1:9, v/v) to obtain the desired product (**2.116**) (90 mg, 12%) as a yellow crystalline solid, m.p. $98-99^\circ\text{C}$.

ν_{max} (KBr, cm^{-1}); 2957, 2928, 1686, 1566, 1070; δ_{H} (CDCl_3); 0.94 (3H, d, J 6.4, CH_3), 1.00 (3H, d, J 6.4, CH_3), 1.01 (3H, s, CH_3), 1.25 (1H, m, CH_2), 1.31 (3H, s, CH_3), 1.62 (1H, d, J 9.4 CH_2), 1.68 (3H, s, CH_3), 1.72 (1H, m, CH), 1.76 (3H, s, CH_3), 1.77 (1, dd, J 13.9, 7.1, CH_2), 1.86 (1H, m, CH_2), 1.93 (2H, m, CH_2), 1.94 (2H, m, CH_2), 1.97 (1H, m, CH), 2.22 (1H, dd, J 13.9, 7.1 CH_2), 2.25 (1H, m, CH_2), 2.26 (1H, d, J 9.4, CH_2), 2.61 (3H, s, CH_3), 2.98 ((1H, m,

CH), 3.41(2H, d, J 7.0, CH₂), 5.12 (1H, t, J 7.0, CH); δ_C (CDCl₃); 18.2 (CH₃), 21.1 (CH₃), 23.1 (CH₃), 23.2 (CH₂), 24.5 (CH₂), 25.4 (CH), 25.6 (CH₃), 25.7 (CH₃), 26.5 (CH₂), 26.8 (CH), 27.1 (CH₃), 27.1(CH₃), 28.2 (CH₂), 32.4 (CH₃), 38.0 (C_q), 39.2 (CH₂), 40.5 (CH), 44.5 (CH₂), 48.8 (CH), 85.0 (C_q), 104.6 (C_q), 108.2 (C_q), 118.2 (C_q), 121.2 (CH), 131.4 (C_q), 163.4 (C_q), 166.1 (C_q), 166.5 (C_q), 204.1(C_q).

Synthesis of 5,7-dihydroxy-3',3'-dimethyl-2',4'-methanospiro(chroman-2,1'-cyclohexan)-4-one (2.117)

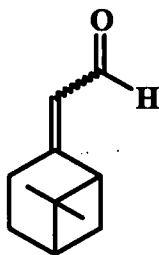


Oxalyl chloride (7.3 g, 58 mmol) was added dropwise to acid **2.121** (3.5 g, 19 mmol) in benzene (5 ml), and stirred for 5 h at room temperature. The excess oxalyl chloride and benzene was removed *in vacuo*. The crude product was dissolved in dry diethyl ether (35 ml), added phloroglucinol (2.0 g, 15 mmol) and ZnCl₂ (2.0 g, 15 mmol) and stirred at room temperature for 2 h. The reaction mixture was poured in to ice cooled solution of 10% aqueous NaHCO₃ (100 ml). The product was extracted with diethyl ether, washed with water and concentrated *in vacuo*. The crude product and K₂CO₃ (5.0 g) in ethanol (50 ml) were heated to reflux for 10 h. On cooling, the solid was filtered, washed with diethyl ether and solvent evaporated *in vacuo*. The

residue was purified by dry column flash chromatography on silica gel using a mixture of diethyl ether:petroleum ether (1:4, v/v) to obtain the title compound **2.117** (3.2 g, 57%) as a white solid.

ν_{max} (KBr, cm^{-1}); 3580, 3460, 3060, 2960, 1705, 1635; δ_{H} (CDCl_3); 1.09 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.48-1.17 (1H, m, CH), 1.78-2.32 (7H, m, $2 \times \text{CH}_2$), 2.71 (1H, d, 17.0, CH_2), 2.78 (1H, d, J 17.0, CH_2), 5.86 (1H, d, 2.1, Ar), 5.93 (1H, d, J 2.1, Ar-H); δ_{C} (CDCl_3); 22.9 (CH_3), 23.6 (CH_2), 26.6 (CH_2), 27.2 (CH_3), 28.7 (CH_2), 38.3 (C_q), 40.5 (CH), 47.2 (CH), 47.8 (CH_2), 86.5 (C_q), 95.9 (CH), 96.0 (CH), 102.8 (C_q), 161.9 (C_q), 163.7 (C_q), 165.4 (C_q), 197.5 (C_q).

Synthesis of 6,6-dimethylbicyclo(3.1.1)heptan-2-ylideneacetaldehyde (**2.120**)

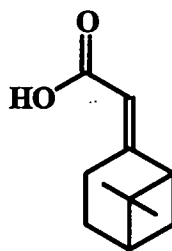


To a stirring solution of oxalyl chloride (5.0 g, 39 mmol) in dichloromethane (100 ml), at -78°C was added a solution of DMSO (6.18 g, 80 mmol) in dichloromethane (50 ml) over 10 min. The stirring was continued for another 20 min and (-) nopol (**2.119**) (5.0 g, 36 mmol) in dichloromethane (50 ml) was added over 10 min. After an additional 30 min with stirring, Et_3N (19.7

ml, 195 mmol) was added over 10 min. The reaction mixture was warmed to room temperature and added water (100 ml) and the organic layer was separated and aqueous layer was extracted with dichloromethane (3 × 100 ml). The combined organic extract was dried over MgSO₄ and concentrated *in vacuo*. The crude product was purified by column chromatography over silica gel using a mixture of petroleum ether:diethyl ether (99:1, v/v) afford the title compound (2.120) (4.5 g, 90%) as a colourless liquid.

ν_{\max} (CHCl₃, cm⁻¹); 2956, 2868, 1614, 1469, 1389; δ_{H} (CDCl₃); 0.77 (3H, s, CH₃), 0.78 (3H, s, CH₃), 1.30 (3H, s, CH₃), 1.35 (3H, s, CH₃), 1.47 (2H, m, CH₂), 1.97 (4H, m, 2 × CH₂), 2.01 (2H, m, CH₂), 2.45 (2H, m, CH₂), 2.61 (1H, t, *J* 6.4, CH), 2.84 (1H, m, CH), 3.19 (1H, dd, *J* 8.8, 8.9, CH), 3.59 (1H, t, *J* 5.9, CH), 5.70 (1H, t, *J*, 2.5, CH), 9.98 (1H, d, *J*, 8.1, CHO); δ_{C} (CDCl₃); 20.3 (CH), 21.7 (CH₃), 23.1 (CH₂), 25.7 (CH₂), 39.9 (CH₂), 40.0 (CH₂), 45.7 (C_q), 53.4 (CH), 124.1 (CH), 172.0 (C_q), 189.1 (C_q).

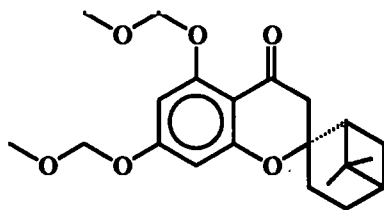
Synthesis of (*E*)-6-6-dimethylbicyclo (3.1.1) heptan-2-ylideneacetic acid (2.121)



To a rapidly stirring solution of the aldehyde 2.120 (4.0 g, 22 mmol), AgNO_3 (4.5 g, 23 mmol) in ethanol (35 ml) and water (6.3 ml) was added slowly a solution of NaOH (0.5 g, 12 mmol) in water (2 ml). The reaction was continued at room temperature for 20 h. The dark black mixture was filtered and the residue was washed with ethanol and water (30 ml, each). The filtrate was extracted with diethyl ether and acidified with 10% H_2SO_4 until pH is five. The aqueous layer was extracted with diethyl ether and combined organic layer was concentrated *in vacuo* to give the title compound (2.121) (3.8 g, 88 %) as a viscous liquid.

ν_{max} (CHCl_3 , cm^{-1}); 3020, 1670, 1620; δ_{H} (CDCl_3); 0.78 (3H, s, CH_3), 1.32 (3H, s, CH_3), 1.47 (2H, m, CH_2), 1.97 (4H, m, $2 \times \text{CH}_2$), 2.61 (1H, t, CH), 3.21 (1H, dd, J 8.2, 8.9, CH), 9.40 (1H, br s, OH); δ_{C} (CDCl_3); 20.5 (CH), 21.8 (CH_3), 23.1 (CH_2), 23.2 (CH_3), 25.7 (CH_2), 39.8 (CH), 45.6 (C_q), 53.4 (CH_2), 112.6 (CH), 172.3 (C_q), 172.9 (C_q).

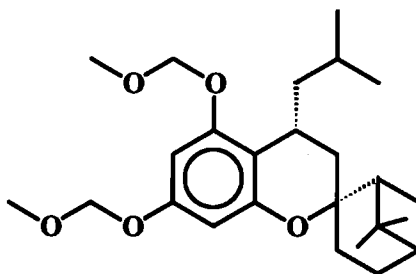
Synthesis of 5,7-dimethoxymethoxy-3,3-dimethyl-2,4-methanospiro(chroman-2,1-cyclohexan)-4-one (2.124)



A solution of chromanone **2.117** (1.5 g, 5.2 mmol), MOMCl (1.0 g, 6 mmol) and freshly dried K_2CO_3 (2.5 g) in dry acetone was heated to reflux for 2 h. The residue of K_2CO_3 was removed by filtration and the solvent concentrated *in vacuo*. The crude mixture was purified by dry column flash chromatography to afford the title compound (**2.124**) (1.5 g, 76%) as an oil.

ν_{\max} ($CHCl_3$, cm^{-1}); 1705, 1630; δ_H ($CDCl_3$); 1.06 (3H, s, CH_3), 1.16 (3H, s, CH_3O), 1.48-1.72 (1H, m, CH), 1.78-2.23 (6H, m, $2 \times CH_2$), 2.72 (1H, d, J 17.0, CH_2), 2.79 (1H, d, J 17.0, CH_2), 3.47 (3H, s, CH_3), 3.52 (3H, s, CH_3), 5.16 (2H, s, CH_2), 5.25 (2H, s, CH_2), 6.24 (1H, d, J 2.2 ArH), 6.26 (1H, d, J 2.2 ArH); δ_C ($CDCl_3$); 22.8 (CH_3), 23.4 (CH_2), 26.6 (CH_2), 27.2 (CH_3), 28.8 (CH_2), 38.3 (C_q), 40.5 (CH), 47.3 (CH), 47.9 (CH_2), 56.3 (CH_2), 56.6 (CH_2), 86.5 (C_q), 93.9 (CH_2), 94.0 (CH_2), 95.8 (CH), 97.2 (CH), 197.2 (C_q).

Synthesis of 5,7-dimethoxymethyl-4-isobutyl-3',3'-dimethyl-2',4'-methanospiro-(chroman-2,1'-cyclohexane) (2.125)

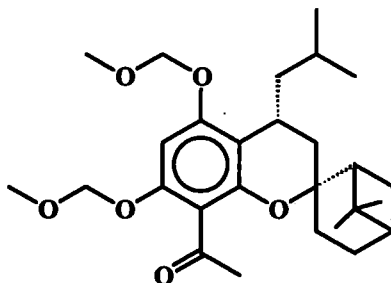


The chromanone **2.124** (1.2 g, 3.1 mmol) in dry THF (15 ml), was treated with isobutyl magnesium chloride (1 ml) at -20°C under N_2 . The reaction mixture was warmed to room temperature over 2 h. After this time 2N HCl (10 ml) was added, extracted with diethyl ether (3×30 ml) and concentrated *in vacuo*. The resulting crude oil was hydrogenated using Pd/C (10%) (0.5 g) in ethanol (15 ml) under H_2 for 3 h at room temperature. The Pd/C was filtered and filtrate concentrated *in vacuo*. The crude product was purified by dry column flash chromatography over silica gel using a mixture of diethyl ether:petroleum ether (1:4, v/v) to afford the title compound (**2.125**) (1.1 g, 90 %) as a mixture of isomers in 9:1 ratio.

δ_{H} (CDCl_3); 0.93 (3H, d, J 6.5, CH_3), 1.00 (3H, d, J 6.5, CH_3), 1.03 (3H, s, CH_3), 1.26 (1H, m, CH_2), 1.30 (3H, s, CH_3), 1.63 (1H, d, J 9.4 CH_2), 1.72 (1H, m, CH), 1.77 (1, dd, J 13.9, 7.1, CH_2), 1.87 (1H, m, CH_2), 1.92 (2H, m, CH_2), 1.94 (2H, m, CH_2), 1.98 (1H, m, CH), 2.23 (1H, dd, J 13.9, 7.1 CH_2), 2.25 (1H, m, CH_2), 2.27 (1H, d, J 9.4, CH_2), 2.97 ((1H, m, CH), 3.47 (3H, s, CH_3),

3.52 (3H, s, CH₃), 5.16 (2H, s, CH₂), 5.25 (2H, s, CH₂), 6.19 9 (1H, d, *J* 2.0, Ar), 6.30 (1H, d, *J* 2.0, Ar-H); δ_c (CDCl₃); 21.0 (CH₃), 23.2 (CH₃), 24.8 (CH₂), 24.9 (CH₃), 25.8 (CH), 26.6 (CH₂), 26.9 (CH), 27.2 (CH₃), 28.3 (CH₂), 38.2 (C_q), 39.1(CH₂), 40.5 (CH), 44.5 (CH₂), 48.9 (CH), 56.3 (CH₃), 56.6 (CH₃), 85.0 (C_q), 93.9 (CH₂), 94.0 (CH₂), 95.9 (CH), 97.4 (CH), 118.6 (C_q), 163.0 (C_q), 163.4 (C_q), 166.5 (C_q).

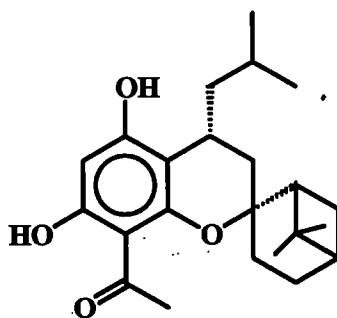
Synthesis of 5,7-dimethoxymethyl-8-oxoethyl-4-isobutyl-3',3'-dimethyl-2',4'-methanospiro-(chroman-2-,1'-cyclohexane) (2.126)



To a solution of TiCl₄ (0.5 ml) and AcCl (164 mg) at 0 °C under N₂ was added the chroman **2.125** (1.0 g, 2.3 mmol) in benzene (10 ml). The mixture was stirred at room temperature for 2 h and added 2N HCl (20 ml). The organic layer was diluted with diethyl ether (25 ml), washed with saturated NaHCO₃, brine, water and then dried over MgSO₄ and concentrated *in vacuo*. The crude product was purified by dry column chromatography using a mixture of diethyl ether:petroleum ether (1:9, v/v) to afford title compound (**2.126**) (1.05 g, 95 %) as an oil.

δ_H (CDCl₃); 0.93 (3H, d, *J* 6.5, CH₃), 1.00 (3H, d, *J* 6.5, CH₃), 1.02 (3H, s, CH₃), 1.26 (1H, m, CH₂), 1.31 (3H, s, CH₃), 1.62 (1H, d, *J* 9.4 CH₂), 1.73 (1H, m, CH), 1.77 (1, dd, *J* 13.9, 7.1, CH₂), 1.87 (1H, m, CH₂), 1.93 (2H, m, CH₂), 1.94 (2H, m, CH₂), 1.97 (1H, m, CH), 2.23 (1H, dd, *J* 13.9, 7.1 CH₂), 2.25 (1H, m, CH₂), 2.27 (1H, d, *J* 9.4, CH₂), 2.61 (3H, s, CH₃), 2.97 ((1H, m, CH), 3.46 (3H, s, CH₃), 3.52 (3H, s, CH₃), 5.16 (2H, s, CH₂), 5.25 (2H, s, CH₂), 6.30 (1H, d, Ar-H); δ_C (CDCl₃); 21.2 (CH₃), 23.1 (CH₃), 24.6 (CH₂), 25.0 (CH₃), 25.6 (CH), 26.6 (CH₂), 26.9 (CH), 27.0 (CH₃), 27.1(CH₃), 28.1(CH₂), 31.0 (CH₃), 38.3 (C_q), 39.0 (CH₂), 40.5 (CH), 44.5 (CH₂), 48.8 (CH), 56.2 (CH₃), 56.4 (CH₃), 85.1 (C_q), 94.0 (CH₂), 94.2 (CH₂), 98.2 (CH), 108.2 (C_q), 118.8 (C_q), 163.4 (C_q), 163.5 (C_q), 165.6 (C_q), 204.1(C_q).

Synthesis of 5,7-dihydroxy-8-oxoethyl-4-isobutyl-3',3'-dimethyl-2',4'-methanospiro-(chroman-2-,1'-cyclohexane) (2.127)



To a cold solution of **2.126** (1.0 g, 2.1 mmol) in methanol (15 ml) was added 10% aq HCl (5 ml). The mixture was heated to reflux for 30 min, poured in to cold water (20 ml) and extracted with diethyl ether (3 × 20 ml). The combined organic extract was washed with water (20 ml), saturated aqueous NaHCO₃

solution (20 ml), then dried over MgSO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography using a mixture of diethyl ether:petroleum ether (1:1, v/v) to afford the title compound (**2.127**) (750 mg, 92%) as a off white crystalline solid, m.p. 98-99 °C.

ν_{max} (KBr, cm^{-1}); 3230, 2950, 2730, 1640, 1610, 1360; δ_{H} (CDCl_3); 0.93 (3H, d, J 6.5, CH_3), 1.00 (3H, d, J 6.5, CH_3), 1.02 (3H, s, CH_3), 1.25 (1H, m, CH_2), 1.31 (3H, s, CH_3), 1.62 (1H, d, J 9.4 CH_2), 1.73 (1H, m, CH), 1.77 (1, dd, J 13.9, 7.1, CH_2), 1.87 (1H, m, CH_2), 1.93 (2H, m, CH_2), 1.94 (2H, m, CH_2), 1.97 (1H, m, CH), 2.21 (1H, dd, J 13.9, 7.1 CH_2), 2.27 (1H, m, CH_2), 2.27 (1H, d, J 9.4, CH_2), 2.62 (3H, s, CH_3), 2.97 ((1H, m, CH), 5.90 (1H, Ar); δ_{C} (CDCl_3); 21.1 (CH_3), 23.1 (CH_3), 24.6 (CH_2), 25.6 (CH), 25.7 (CH_3), 26.6 (CH_2), 26.9 (CH), 27.1 (CH_3), 28.2 (CH_2), 32.0 (CH_3), 38.0 (C_q), 39.1 (CH_2), 40.4 (CH), 44.5 (CH_2), 48.9 (CH), 85.1 (C_q), 96.4 (CH), 108.4 (C_q), 118.5 (C_q), 163.4 (C_q), 163.5 (C_q), 166.3 (C_q), 166.7 (C_q), 203.9(C_q).

REFERENCES

- 1 P. Mathew, 'World Crop Losses', Wood Mackenzie, 1997.
- 2 M. Miyakado, K. Watanabe, and J. Miyamoto, *Phytochemicals for Pest Control*, American Chemical Society, Honolulu, 1997, p.168-182.
- 3 P. Mathew, 'Product Groups Market Values', Wood Mackenzie, 1998.
- 4 G. A. McLaughlin, in 'History of Pyrethrum', ed. J. E. Casida, Academic Press, New York and London, 1973, p. 3-15.
- 5 A. E. Smith and D. M. Secoy, *Chemistry and Industry*, 1981, 12-17.
- 6 H. Dehne and F. Schonbeck, in 'Crop protection - Past and Present', ed. E. C. Oerke and A. Weber, Elsevier, Amsterdam, 1994.
- 7 P. Mathew, 'Global Insecticide Market', Wood Mackenzie, 1997.
- 8 P. Smith, in 'Pesticides in Foodstuffs', ed. G. Best and D. Ruthven, The Royal Society of Chemistry, Cambridge, 1995, p. 62-73.
- 9 R. W. Lemon, *Journal of Agricultural Science*, 1994, 122, 329-333.
- 10 M. B. Isman, *Review of Pesticide Toxicology*, 1995, 3, 1-20.
- 11 N. R. Farnsworth, in 'The Role of Ethnopharmacology in Drug Development', Ciba Foundation Symposium, John Wiley & Sons, 1990, p.2-21.
- 12 M. Grainge and S. Ahmed, 'Handbook of Plants with Pest-Control Properties', John Wiley & Sons, 1988.
- 13 W. S. Bowers, in 'Fourth-Generation Pesticides', ed. D. L. Whitehead and W. S. Bowers, pergamon Press, Oxford, 1983. p. 49.
- 14 C. A. Henrick, in 'Pyrethroids', ed. C. R. A. Godfrey, Marcel Dekker, Inc., Bracknell, 1995, p.63-145.

- 15 H. Staudinger and L. Ruzicka, *Helvetica Chimica Acta*, 1924, **7**, 177.
- 16 M. Elliot, *Synthetic Pyrethroids*, American Chemical Society, San Francisco, 1977, p. 1-28
- 17 M. Elliot and N. Janes, *Chemical Society Reviews*, 1978, **7**, 473-505.
- 18 M. S. Schechter, N. Green, and F. B. Laforge, *Journal of the Chemical Society*, 1949, **71**, 3165-3173.
- 19 N. Matsuo and J. Miyamoto, *Phytochemicals for Pest Control*, American Chemical Society, Honolulu, 1997, p. 182-193.
- 20 M. Matsui, F. B. Laforge, N. Green, and M. S. Schechter, *Journal of the American Chemical Society*, 1952, **74**, 2181.
- 21 M. Elliot, A. W. Farnham, N. F. Janes, and P. H. Needham, *Pesticide Science*, 1974, **5**, 491-496.
- 22 K. Ueda, L. C. Gaughan, and J. E. Casida, *Journal of Agriculture and Food Chemistry*, 1974, **22**, 212-220.
- 23 T. Kato, K. Ueda, and K. Fujimoto, *Journal of Agriculture and Food Chemistry*, 1965, **28**, 914-915.
- 24 Y. Katsuda, *Journal of Pesticide Science*, 1982, **7**, 317-327.
- 25 T. Matsuo, N. Itaya, T. Mizutani, N. Ohno, K. Fujimoto, and Y. Okuno, *Journal of Agriculture and Biological Chemistry*, 1976, **40**, 247.
- 26 M. Elliott, A. W. Farnham, N. F. Janes, P. H. Needham, D. A. Pulman, and J. H. Stevenson, *Nature*, 1973, **246**, 169-170.

- 27 M. Elliott, A. W. Farnham, N. F. Janes, P. H. Needham, and D. A. Pulman, *Nature*, 1974, **248**, 710-711.
- 28 I. Hammann and R. Fuchs, in 'Baythroid, a New Insecticide', Pflanzenschutz Nachrichten Bayer, Leverkusen, 1981, p. 121-151.
- 29 E. McDonald, N. Punja, and A. R. Jutsum, Pests and Diseases, Brighton Crop Protection Conference, Brighton, 1986, p. 199-206.
- 30 N. Ohno, K. Fujimoto, Y. Okuno, T. Mizutani, M. Hirano, N. Itaya, T. Honda, and H. Yoshioka, *Agricultural and Biological Chemistry*, 1974, **38**, 881-883.
- 31 C. A. Henrick, B. A. Garcia, G. B. Staal, D. C. Cerf, R. J. Anderson, K. Gill, H. R. Chinn, and G. K. Kohn, *Pesticide Science*, 1980, **11**, 224-241.
- 32 M. J. Bull, J. H. Davies, R. J. G. Searle, and A. C. Henry, *Pesticide Science*, 1980, **11**, 249-256.
- 33 K. Nanjyo, N. Katsuyama, A. Kariya, T. Yamamura, S. Hyeon, A. Suzuki, and S. Tamura, *Agricultural and Biological Chemistry*, 1980, **44**, 217-218.
- 34 T. Udagawa, S. Numata, K. Oda, S. Shiraishi, K. Kodaka, and K. Nakatani, Recent Advances in the Chemistry of Insect Control, Royal Society of Chemistry, Cambridge, 1984, p. 192-204.
- 35 M. Elliot, A. W. Farnham, N. F. Janes, and B. P. S. Khambay, *Pesticide Science*, 1988, **22**, 231-249.

- 36 R. F. S. Gordon, M. J. Bushell, R. Pascoe, and T. Enoyoshi, British Crop Protection Conference -Pest and Diseases, Brighton, 1992, p. 81-88.
- 37 Y. Yamada, T. Yano, and N. Itaya, *Journal of Pesticide Science*, 1987, 12, 683-688.
- 38 R. W. Addor, in 'Isecticides', ed. C. R. A. Godfrey, Marcel Dekker, Inc.,Bracknell, 1995, p. 1-62.
- 39 G. W. Ware, in 'Pesticides Theory and Application', W. H Freeman & Company, San Francisco, 1978, p. 145-154.
- 40 S. B. Soloway, A. C. Henry, W. D. Kollmeyer, W. M. Padgett, J. E. Powell, S. A. Roman, C. H. Tieman, R. A. Corey, and C. A. Horne, 'Advances in Pesticide Science', ed. H. Geissbuhler, Pergamon Press, New York, 1979, p. 206-217.
- 41 A. Elbert, H. Overbeck, K. Iwaya, and S. Tsuboi, Brighton Crop protection Conference, Surrey, 1990, p. 21-28.
- 42 H. Cheung, S. B. Clarke, and J. D. Beadle, *Journal of Pesticide Science*, 1992, 34, 187-193.
- 43 I. Minamida, K. Iwanaga, T. Tabuchi, H. Uneme, H. Dantsuji, and T. Okauchi, *Journal of Pesticide Science*, 1993, 18, 31-40.
- 44 A. Nakayama, M. Sukekawa, and Y. Eguchi, *Journal of Pesticide Science*, 1997, 51, 157-164.
- 45 E. Stedman and G. Burger, *Journal of the Chemical Society*, 1925, 127, 247.

- 46 A. Stempel and J. A. Aeschlimann, in 'Synthetic Analogues of Physostigmine', ed. F. F. Blickse and R. H. Cox, John Wiley & Sons, New York, 1956.
- 47 W. H. Tisdale and A. L. Flenner, *Industrial and Engineering Chemistry*, 1942, **34**, 501-502.
- 48 J. R. Geiggy, *In Swiss Patent*, 279, 1952.
- 49 G. R. Ferguson and C. C. Alexander, *Journal of Agriculture and Food Chemistry*, 1953, **1**, 888-889.
- 50 K. Gubler, A. Margot, and H. Gysin, *Journal of the Science of Food and Agriculture Suppl.*, 1968, **13**.
- 51 H. L. Haynes, J. A. Lambrech, and H. H. Moorefield, *Contrib, Boyce Thompson Inst.*, 1957, **18**, 507.
- 52 M. J. Kolbezen, R. L. Metcalf, and T. R. Fukuto, *Journal of Agriculture and Food Chemistry*, 1954, **2**, 864-870.
- 53 L. K. Payne, H. A. Stansburg, and M. H. J. Weiden, *Journal of Agriculture and Food Chemistry*, 1966, **14**, 356-365.
- 54 N. Umestu, *Journal of Pesticide Science*, 1986, **11**, 493-503.
- 55 P. J. Jewess, 'Inhibition of Acetylcholinesterase by Trizamate', Shell Research, Sittingbourne, 1993.
- 56 J. I. Fukami, 'Insecticides as Inhibitors of Respiration', ed. H. C. Keyserlingk, A. Jager, and C. Szczepanski, Springer-Verlag, 1985.
- 57 P. R. Jefferies and J. E. Casida, *Natural and Engineered Pest Management Agents*, American Chemical Society, Utah, 1994, p. 131.

- 58 I. N. Pessah, Recent Advances in the Chemistry of Insect Control II, Royal Society of Chemistry, Oxford, 1989, p. 278-296.
- 59 S. L. Ley, A. A. Denholm, and A. Wood, *Natural Products Reports*, 1993, 109-157.
- 60 B. H. Broughton, S. V. Ley, A. M. Z. Slawin, D. J. Williams, and E. D. Morgan, *Journal of Chemical Society Chemical Communications*, 1986, 46.
- 61 J. H. Butterworth and E. D. Morgan, *Chemical communications*, 1968, 23.
- 62 A. J. Mordue and A. Blackwell, *Journal of Insect Physiology*, 1993, 39, 903-924.
- 63 S. Chiu, *Journal of Applied Entomology*, 1989, 107, 185-192.
- 64 M. Miyakado, I. Nakayama, and N. Ohno, *Insecticides of Plant Origin*, American Chemical Society, Toronto, 1988, p. 173-187.
- 65 M. Elliott, W. A. Farnham, N. F. Janes, D. M. Johnson, and D. A. Pulman, *Pesticide Science*, 1987, 18, 191-201.
- 66 J. A. Ottea, G. T. Payne, and D. M. Soderlund, *Journal of Agriculture and Food Chemistry*, 1990, 38, 1724-1728.
- 67 F. Ishibashi, C. Satasook, M. B. Isman, and G. H. N. Towers, *Phytochemistry*, 1993, 32, 307-310.
- 68 L. P. Molleyres and V. Kumar, In Swiss Patent, PCT, Int. Appl. W09604284, Switzerland.

- 69 J. Janprasert, C. Satasook, P. Sukumalanand, D. E. Champagne, M. B. Isman, P. Wiriyaichitra, and G. H. N. Towers, *Phytochemistry*, 1993, **32**, 67-69.
- 70 C. Satasook, M. B. Isman, and P. Wiriyaichitra, *Pesticide Science*, 1992, **36**, 53-58.
- 71 L. Crombie, Recent Advances in the Chemistry of Insect Control II, Oxford, UK, 1989, p. 23-51.
- 72 J. P. Pachlatko, *Chimia*, 1998, **52**, 29-47.
- 73 E. Taniguchi, K. Imamura, F. Ishibashi, T. Matsui, and A. Nishio, *Agricultural and Biological Chemistry*, 1989, **53**, 631-643.
- 74 S. Yamauchi and E. Taniguchi, *Bioscience Biotechnology Biochemistry*, 1992, **56**, 1744-1750.
- 75 M. D. Esposti, A. Ghelli, M. Ratta, D. Cortes, and E. Estornell, *Biochemistry Journal*, 1994, **301**, 161-167.
- 76 M. Londershausen, L. Wolfgang, F. Leib, and H. Moeschler, *Pesticide Science*, 1991, **33**, 427-438.
- 77 J. A. Klocke and I. Kubo, *Entomology Exp. Appl.*, 1982, **32**, 299-301.
- 78 D. K. Murray, A. R. Alford, E. Groden, F. A. Drummond, R. H. Storch, M. D. Bentley, and P. M. Sugathapala, *Journal of Economic Entomology*, 1993, **86**, 1793-1801.
- 79 J. E. Hawkinson and J. E. Casida, Pest Control with Enhanced Environmental Safety, American Chemical Society San Francisco, 1992, p. 126-143.

- 80 E. L. Rice, 'Allelopathy', Academic Press Limited, New York, 1994.
- 81 G. T. Gujar, *Fitoterapia*, 1990, **LXI**, 387-393.
- 82 A. Coghlan, *New Scientist*, 1996, 9.
- 83 B. P. S. Khambay, D. G. Beddie, and M. S. J. Simmonds,
Phytochemical Diversity, Royal Society of Chemistry, Brighton, UK,
1997, p. 166.
- 84 B. P. S. Khambay, D. G. Beddie, D. Batty, I. Denholm, and R. M.
Cahill, *Pesticide Science*, 1997, **50**, 291-296.
- 85 I. K. Gabe, in 'Avermectins and Milbemycins', ed. C. R. A. Godfrey,
Marcel Dekker, Inc., Bracknell, 1995, p. 215-255.
- 86 C. J. Dutton, S. P. Gibson, M. Kinns, A. G. Swanson, and J. Bordner,
Journal of the Chemical Society Perkin Transaction 2, 1995, **2**, 403-
408.
- 87 C. J. Dutton, A. M. Hooper, P. F. Leadlay, and J. Staunton,
Tetrahedron Letters, 1994, **35**, 327-330.
- 88 A. C. Goudie, N. A. Evans, and K. A. F. Gration, *Veterinary
Parasitology*, 1993, **49**, 5-15.
- 89 P. R. Susan and P. A. Joseph, American Chemical Society, 1995, p.
264.
- 90 U. Mothes and K. A. Seitz, *Pesticide Science*, 1982, **13**, 426-441.
- 91 H. Decker, C. Bormann, H. Fiedler, and H. Zahner, *The Journal of
Antibiotics*, 1989, **42**, 230-235.

- 92 R. Jansen, B. Kunze, H. Reichenbach, E. Jurkiewicz, G. Hunsmann, and G. Hofle, *Leibigs Annalen der Chemie*, 1992, 357-359.
- 93 H. Gerhard, N. Bedorf, and P. Johannes, in 'Thiangazole, Its Preparation, Compositions and Use Thereof', W.O pat.A/92/11258, Canada, 1992.
- 94 J. Ehrler and S. Farooq, *Synlett Letters*, 1994, 702-704.
- 95 H. A. Kirst, K. H. Michel, J. W. Martin, L. C. Creemer, E. H. Chio, R. C. Yao, and G. D. Thompson, *Tetrahedron Letters*, 1991, 32, 4839-4842.
- 96 C. V. Deamics, J. E. Dripps, C. J. Hatton, and L. L. Karr, *Phytochemicals for Pest Control*, American Chemical Society Honolulu, 1997, p. 144-154.
- 97 D. A. Evans and W. C. Black, *Journal of the American Chemical Society*, 1992, 114, 2260-2262.
- 98 V. L. Salgado, G. B. Watson, and J. J. Sheets, *Cotton Insect Research and Control Conference*, Indianapolis, 1997, p. 1082-1087.
- 99 H. Nakamura, K. Shiomi, H. Iinuma, H. Naganawa, and T. Obata, *The Journal of Antibiotics*, 1987, 899-903.
- 100 R. W. Addor, T. J. Babcock, B. C. Black, D. G. Brown, R. E. Diehl, and J. A. Furch, *Synthesis and Chemistry of Agrochemicals III*, American chemical Society, Washinton, 1992, p. 283.

- 101 J. R. Lewis, in 'Chemistry and Chemical Taxonomy of the Rutales',
ed. P. G. Waterman and M. F. Grondon, Academic press Inc. p.301-
318, London, 1983.
- 102 D. M. A. Jayaweera, 'Medicinal Plants used in Ceylon', National
Science Council of Sri Lanka, 1982, p.3.
- 103 V. Kumar, Karunaratne.V., M. R. Sanath, K. Meegalle, and J. K.
Macleod, *Phytochemisrry*, 1990, **29**, 243-245.
- 104 T. Wu, M. Wang, and T. Jong, *Journal of Natural Products*, 1989, **52**,
1284-1289.
- 105 F. N. Lahey and R. Hutchins, Symposium on Phytochemistry, Hong
Kong, 1961, p. 121-122.
- 106 T. R. Govindachari, S. S. Sathe, and N. Viswanathan, *Indian Journal
of Chemistry*, 1969, **7**, 873-875.
- 107 J. Banerji, R. N. Rej, and A. Chatterjee, *Indian Journal of Chemistry*,
1973, **11**, 693-694.
- 108 V. Kumar, V. Karunaratne, K. Meegalle, and M. R. Sanath,
Phytochemistry, 1989, **28**, 1278-1279.
- 109 V. Kumar, L. B. De Silva, W. M. Herath, C. Liyanage, V. U. Ahmad,
and A. Sultana, *Phytochemistry*, 1991, **30**, 1709-1710.
- 110 L. B. De Silva, U. L. L. De Silva, M. Mahendran, and R. Jennings,
Phytochemistry, 1979, **18**, 1255-1256.
- 111 R. C. Seccombe and C. H. L. Kennard, *Journal of Applied
Crystallography*, 1974, **7**, 512-513.

- 112 H. Wagner and J. Burghart, *Helvetica Chimica Acta*, 1981, **64**, 283.
- 113 C. Seguneau, P. Richomme, J. Bruneton, and J. Pusset, *Helvetica Chimica Acta*, 1992, **75**, 2283-2288.
- 114 C. Seguneau, P. Richomme, J. Bruneton, and P. Meadows, *Heterocycles*, 1994, **38**, 181-187.
- 115 A. P. Dantanarayana, N. S. Kumar, U. S. Sultanbawa, and S. Balasubramaniam, *Journal of the Chemical Society Perkin Transaction I*, 1981, 2717-2723.
- 116 A. P. Dantanarayana, N. S. Kumar, P. M. Muthukuda, and S. Balasubramaniam, *Phytochemistry*, 1983, **22**, 473-474.
- 117 A. P. Dantanarayana, N. S. Kumar, P. M. Muthukuda, and M. I. M. Wazeer, *Phytochemistry*, 1982, **21**, 2065-2068.
- 118 R. Bruning and H. Wagner, *Phytochemistry*, 1978, **17**, 1821-1858.
- 119 Y. Q. Tu, *Journal of the Chemical Society Perkin Transaction I*, 1991, 425-427.
- 120 A. Getahun and A. D. Krikorian, *Economic Botany*, 1973, **27**, 353.
- 121 J. K. R. R. Samarasekera, 'Insecticidal Natural Products from Sri Lankan Plants', Ph.D Thesis, Open University, Milton Keynes, 1997, p. 58-91, 207.
- 122 O. Shiota, H. Morita, K. Takeya, and H. Itokawa, *Heterocycles*, 1994, **38**, 383-389.
- 123 D. Waldi, 'Spray Reagents for Thin-layer Chromatography', ed. E. Stahl, Springer-Verlag, 1969, p. 483-502.

- 124 A. G. Gonzalez, I. A. Jimenez, A. G. Ravelo, J. Sazatornil, and I. L. Bazzocchi, *Tetrahedron*, 1993, **49**, 697-702.
- 125 K. Yamada, Y. Shizuri, and Y. Hirata, *Tetrahedron*, 1978, **34**, 1915-1918.
- 126 L. F. Johnson and W. C. Jankowski, '13Carbon NMR Spectra : A Collection of Assigned, Coded, and Indexed Spectra', John Wiley and Sons, New York, 1972.
- 127 H. Wagner, R. Bruning, H. Lotter, and A. Jones, *Tetrahedron Letters*, 1977, 125-128.
- 128 N. H. Lajis and Z. Mahmud, *Journal of Natural Products*, 1991, **54**, 612-614.
- 129 Z. Mahmud, M. N. Khan, and N. H. Lajis, *Journal of Natural Products*, 1992, **55**, 533-535.
- 130 E. D. Becker and M. Beroza, *Tetrahedron Letters*, 1962, 157-163.
- 131 A. Pelter and R. S. Ward, *Tetrahedron*, 1976, **32**, 2783-2788.
- 132 P. Budowski, *Journal of the American Oil Chemists Society*, 1964, **41**, 280-285.
- 133 K. K. Purushothaman, K. Duraiswamy, J. D. Connolly, and D. Rycroft, *Phytochemistry*, 1985, **24**, 2349-2354.
- 134 J. F. Ayafor, B. L. Sondengam, J. D. Connolly, D. S. Rycroft, and J. I. Okogun, *Journal of the Chemical Society Perkin Transaction I*, 1980, 1750-1753.

- 135 D. A. Mulholland, R. Osborne, S. L. Robberts, and D. A. H. Taylor, *Phytochemistry*, 1994, **37**, 1417-1420.
- 136 K. W. M. Zuurbier, S. Fung, J. J. C. Scheffer, and R. Verpoorte, *Phytochemistry*, 1995, **38**, 77-82.
- 137 A. J. Birch, J. L. Willis, R. O. Hellyer, and M. Salahud-Din, *Journal of the Chemical Society*, 1966, 1337.
- 138 K. B. G. Torsell, in 'The Polyketide Pathway', Apotekarsocieteten, Sweden, 1997, p. 199-202.
- 139 E. L. Ghisalberti, *Phytochemistry*, 1996, **41**, 7-22.
- 140 K. Lal, E. A. Zarate, W. J. Youngs, and R. G. Salomon, *Journal of Organic Chemistry*, 1988, **53**, 3673-3680.
- 141 I. P. Singh and H. Etoh, *Bioscience Biotechnology Biochemistry*, 1995, **59**, 2330-2332.
- 142 J. Jakupovic, J. Kuhnke, A. Schuster, M. A. Metwally, and F. Bohlmann, *Phytochemistry*, 1986, **25**, 1133-1142.
- 143 J. Kuhnke and F. Bohlmann, *Tetrahedron Letters*, 1985, **20**, 3955-3958.
- 144 Olah, 'Friedel Crafts and Related Reactions', Interscience, New York, 1963.
- 145 H. Jayasuriya, J. K. Baker, M. A. Clark, and J. D. McChesney, *Pharmaceutical Research*, 1991, **8**, 1372-1376.
- 146 R. Huebner, *Chemistry Ber.*, 1957, **90**, 2870-2874.

- 147 K. Glusenkamp and G. Buchi, *Journal of Organic Chemistry*, 1986, **51**, 4481-4483.
- 148 A. De Renzi, A. Panunzi, A. Saporito, and A. Vitagliano, *Journal of the Chemical Society Perkin Transaction II*, 1983, 993-996.
- 149 D. N. Sarma, P. Baruah, U. C. Pandey, and R. P. Sharma, *Tetrahedron Letters*, 1984, **25**, 5581-5584.
- 150 A. C. Jain, P. Lal, and T. R. Seshadri, *Tetrahedron*, 1970, **26**, 2631-2635.
- 151 A. C. Jain and M. K. Zutshi, *Tetrahedron Letters*, 1971, 3179-3182.
- 152 R. G. Dushin, *Journal of the American Chemical Society*, 1992, **114**, 655-659.
- 153 D. Crich, H. Dyker, and J. R. Harris, *Journal of Organic Chemistry*, 1989, 257-259.
- 154 D. L. Hughes, R. A. Reamer, J. J. Bergan, and E. J. J. Grabowski, *Journal of the American Chemical Society*, 1988, **110**, 6487-6491.
- 155 O. Mitsunobu, *Synthesis*, 1981, 1-28.
- 156 H. Knolker and W. Frohner, *Tetrahedron Letters*, 1997, **38**, 4051-4054.
- 157 D. R. Crump, R. W. Franck, R. Gruska, A. A. Ozorio, M. Pagnotta, G. J. Siuta, and J. G. White, *Journal of Organic Chemistry*, 1977, **42**, 105-108.
- 158 G. Burkhardt, H. Becker, M. Grubert, J. Thomas, and T. Eicher, *Phytochemistry*, 1994, **37**, 1593-1597.

- 159 M. Tsukayama, M. Kikuchi, and S. Yoshioka, *Chemistry Letters*, 1993, 1895-1898.
- 160 E. B. Merkushev, *Synthesis*, 1987, 923-936.
- 161 M. C. Carreno, J. L. G. Ruano, G. Sanz, M. A. Toledo, and A. Urbano, *Tetrahedron Letters*, 1996, 37, 4081-4084.
- 162 A. L. Silva, B. Quiroz, and A. Maldonado, *Tetrahedron Letters*, 1998, 39, 2055-2050.
- 163 X. Bu, L. Zhao, and Y. Li, *Synthesis*, 1997, 1246-1248.
- 164 R. D. H. Murray, M. M. Ballantyne, and K. P. Mathai, *Tetrahedron Letters*, 1970, 243-244.
- 165 G. Casiraghi, G. Casnati, G. Puglia, and G. Sartori, *Synthesis*, 1980, 124-125.
- 166 G. Casiraghi, G. Casnati, A. Pochini, G. Puglia, R. Ungaro, and G. Sartori, *Synthesis*, 1981, 143-146.
- 167 K. Bock, N. Jacobson, and B. Terem, *Journal of the Chemical Society Perkin Transaction I*, 1986, 659-664.
- 168 P. S. Kalsi, J. Singh, W. D. Crow, and B. R. Chhabra, *Phytochemistry*, 1987, 26, 3367-3369.
- 169 K. Osawa and H. Yasuda, *Journal of Natural Products*, 1996, 59, 823-827.
- 170 T. Meikle and R. Stevens, *Journal of the Chemical Society Perkin Transaction I*, 1978, 1303-1312.

- 171 Q. Cheng and J. K. Snyder, *Journal of Organic Chemistry*, 1988, **53**, 4562-4567.
- 172 H. Satoh, H. Etoh, N. Watanabe, H. Kawagishi, K. Arai, and K. Ina, *Chemistry Letters*, 1992, 1917-1920.
- 173 D. G. Beddie, 'Isolation, Characterisation and Synthesis of Insecticidal Natural Products of the Myrtaceae Family', University of Nottingham, Nottingham, 1998, p. 123-125.
- 174 D. G. Beddie, 'Isolation, Characterisation and Synthesis of Insecticidal Natural Products of the Myrtaceae Family', University of Nottingham, Nottingham, 1998, p. 63-65.
- 175 D. G. Beddie, 'Isolation, Characterisation and Synthesis of Insecticidal Natural Products of the Myrtaceae Family', University of Nottingham, Nottingham, 1998, p. 105-106.
- 176 D. G. Beddie, 'Isolation, Characterisation and Synthesis of Insecticidal Natural Products of the Myrtaceae Family', University of Nottingham, Nottingham, 1998, p. 107-108.
- 177 K. Lal, E. A. Zarate, W. J. Youngs, and R. G. Salomon, *Journal of the American Chemical Society*, 1986, **108**, 1311-1312.
- 178 K. Lal, S. Ghosh, and R. G. Salomon, *Journal of Organic Chemistry*, 1987, **52**, 1072-1078.
- 179 K. Lal, E. A. Zarate, W. J. Youngs, R. G. Salomon, and S. M. Mazza, *Journal of the American Chemical Society*, 1988, **110**, 5213-5214.

- 180 S. Koser, H. M. R. Hoffmann, and D. J. Williams, *Journal of Organic Chemistry*, 1993, **58**, 6163-6165.
- 181 I. R. Aukrust and L. Skattebol, *Acta Chemica Scandinavica*, 1996, **50**, 132-140.
- 182 M. Majewski and G. Bantle, *Tetrahedron Letters*, 1989, **30**, 6653-6656.
- 183 P. J. Jewess, B. P. S. Khambay, K. Chamberlain, and A. L. Devonshire, 9 th International Congress Pesticide Chemistry, London, 1988, p. 24.
- 184 J. R. J. Pare, M. Sigouin, S. Bagot, J. Lapointe, and S. Hyacinthe, in 'Microwave-Assited Natural Products Extraction', U.S. patent, p.2-6, Canada, 1991.
- 185 K. Ganzler, A. Salgo, and A. Valko, *Journal of Chromatography*, 1986, **371**, 299-306.
- 186 L. M. Harwood, *Aldricha Chimica Acta*, 1985, **18**, 25.
- 187 J. C. Coll and B. F. Bowden, *Journal of Natural Products*, 1986, **49**, 934-936.
- 188 W. C. Still, M. Khan, and A. Mitra, *Journal of Organic Chemistry*, 1978, **43**, 2923-2925.
- 189 Vogel, in 'Isolation and Purification Process - Chromatography', ed. B. S. Furniss, A. J. Hanaford, P. W. G. Smith, and A. R. Tatchell, Longman Scientific and Technical, UK, 1989.

- 190 M. R. Cahill and B. Hackett, Proceedings Brighton Crop Protection, Brighton, 1992, p. 251-256.
- 191 T. J. Dennehy, A. W. Farnham, and I. Denholm, *Pesticide Science.*, 1993, **39**, 47-54.
- 192 J. L. Mc Laughlin, in 'Crown Gall Tumours on Potato Discs and Brine Shrimp Lethality: Two Simple Bioassays for Higer Plant Screening and Fractionation', ed. K. Hostettmann, London, 1991.
- 193 B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobson, D. E. Nichols, and J. L. Mc Laughlin, *Planta Medica*, 1982, **45**, 31.

APPENDIX I

Bioassay Procedures

(I) INSECTICIDAL BIOASSAY

(a) Housefly (*Musca domestica*)

The houseflies were treated by the topical application method. Female flies (30 per concentration) were dosed on the thorax with 1 μ l of the test solution. The treated flies were maintained at 20 ± 1 °C and fed on a cotton wool plug soaked in a sucrose solution. Line tests used 6 dose rates per compound, two replicates of 15 flies for each. The percentage of mortality was assessed 24 and 48 h after treatment. The LD₅₀ values were calculated in micrograms of test compound per fly.

(b) Dimondback moth (*Plutella xylostella*)

The diamondback moth larvae were treated by topical application method. Fifth instar larvae (30 per concentration) were dosed with 0.5 μ l of the test compound in acetone. Treated larvae were maintained at 22 °C and mortality was assessed as failure to pupate 5 days later. Line tests used 5 dose rates per compound, three replicates of 10 larvae for each. The LD₅₀ values were calculated as for houseflies.

(c) Whitefly (*Bemisia tabaci*)

White flies were treated using a vial confinement method. The test was carried out using three different strains of whitefly, one susceptible and two resistant to

pyrethroid insecticides. The susceptible strain (SUD-S) was collected in Sudan in 1978 from cotton. The resistant strains, Ned 3 and USA-B, were collected from Gerbera, Netherlands in 1992 and from Poinsettia, Southern USA in 1985, respectively.

A solution of test compound in acetone (100 µl) was placed in 10 ml glass vials and evaporated with rotation to deposit a film of the compound. Adult whiteflies (30 per replicate, 3 replicates per dose, 5, 7 dose levels) were confined inside the vial for 1 h and then transferred onto untreated cotton leaf discs which were kept moist on a bed of agar gel. The temperature was maintained at 25 °C and mortality assessed after 48 h. LC₅₀ values (in ppm) were calculated as above.

(d) Mites (*Tetranychus urticae*)

The mites were treated by microimmersion of topical application method using three different strains of mites, one susceptible and two resistant to bifenthrin and carbaryl. The susceptible strain (GSS) was supplied by Schering, AG, Berlin. The resistant strain, NYR-Bif-1000 was provided by the Department of Entomology, Cornell University, New York, having subjected a field strain to selection with bifenthrin. The resistant strain, UK-S Carb-600 was obtained by applying selection with carbaryl to the UK-S strain provided by Shell Research Limited, Sittingbourne, UK.

Adult female mites (25 per replicate, 3 replicates, 5, 6 dose rates) were immersed in 35 µl of an acetone:water (1:4) solution of the test compound for 30 seconds.

The treated mites were maintained at $21 \pm 2^\circ\text{C}$ and mortality was assessed 72 h after treatment. Mites exhibiting repetitive (non-reflex) movement of more than one locomotory appendage after this period were recorded as alive. The LC_{50} values (in ppm) were calculated as above.

(II) ANTIBACTERIAL BIOASSAY

10 μl of compounds at 100 ppm, 10 ppm and 1 ppm in acetone solution were introduced into wells of bacteria seeded agar. Only compounds showing activity at 100 ppm were tested at lower concentration. After inoculation of the agar the Petri dishes were left to incubate for 48 hours at 25°C . Inhibition zones were measured to the nearest mm using vernier calipers, two measurement per replicate. The experiment was run three times with 10 replicates per run ($n = 30$).

APPENDIX II

^1H and ^{13}C NMR spectra of compounds 1 - 6

29.77

12HE8

8.02

1.00

1.96

0.07

¹H-NMR of compound 1

30 SEP 94 10:11:45

DFILE 12HE801

OBNUC 1H

PROPT 32758

FRECU 3000.0 Hz

SCANS 10

SLVNT CDCL3

Temp: 50°C

294

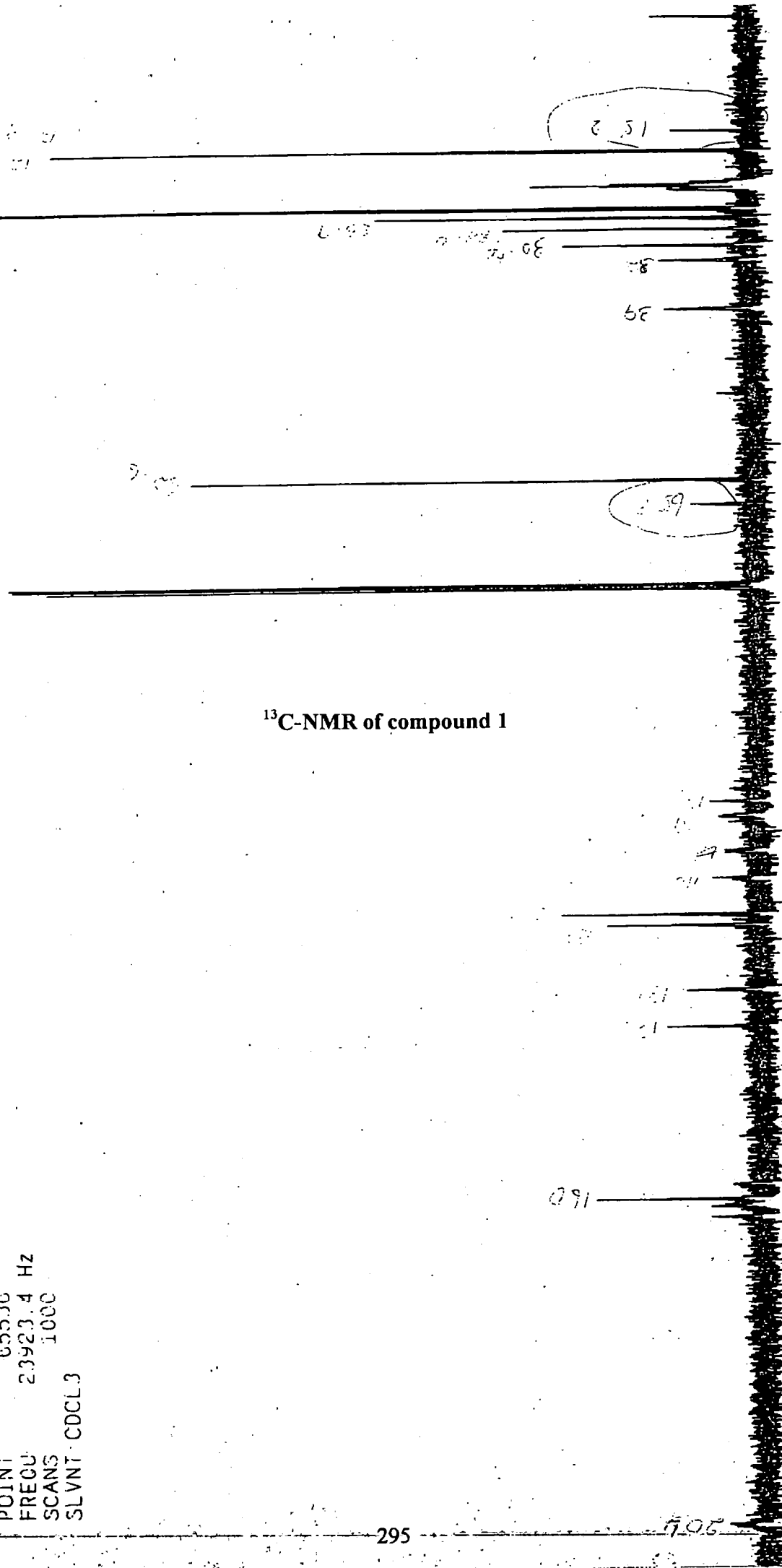
2.27

12HE8

30-SEP-94 15:57:40

DFILE 12HE803
OBNUC 13C
POINT 6553G
FREQ 23923.4 Hz
SCANS 1000
SLVNT CDCL3

¹³C-NMR of compound 1



ppm 0 1 2 3 4 5 6 7 8 9

¹H-NMR of compound 2

4.7885
8.3641

3.7693

10.1511

12.0153

14.1270

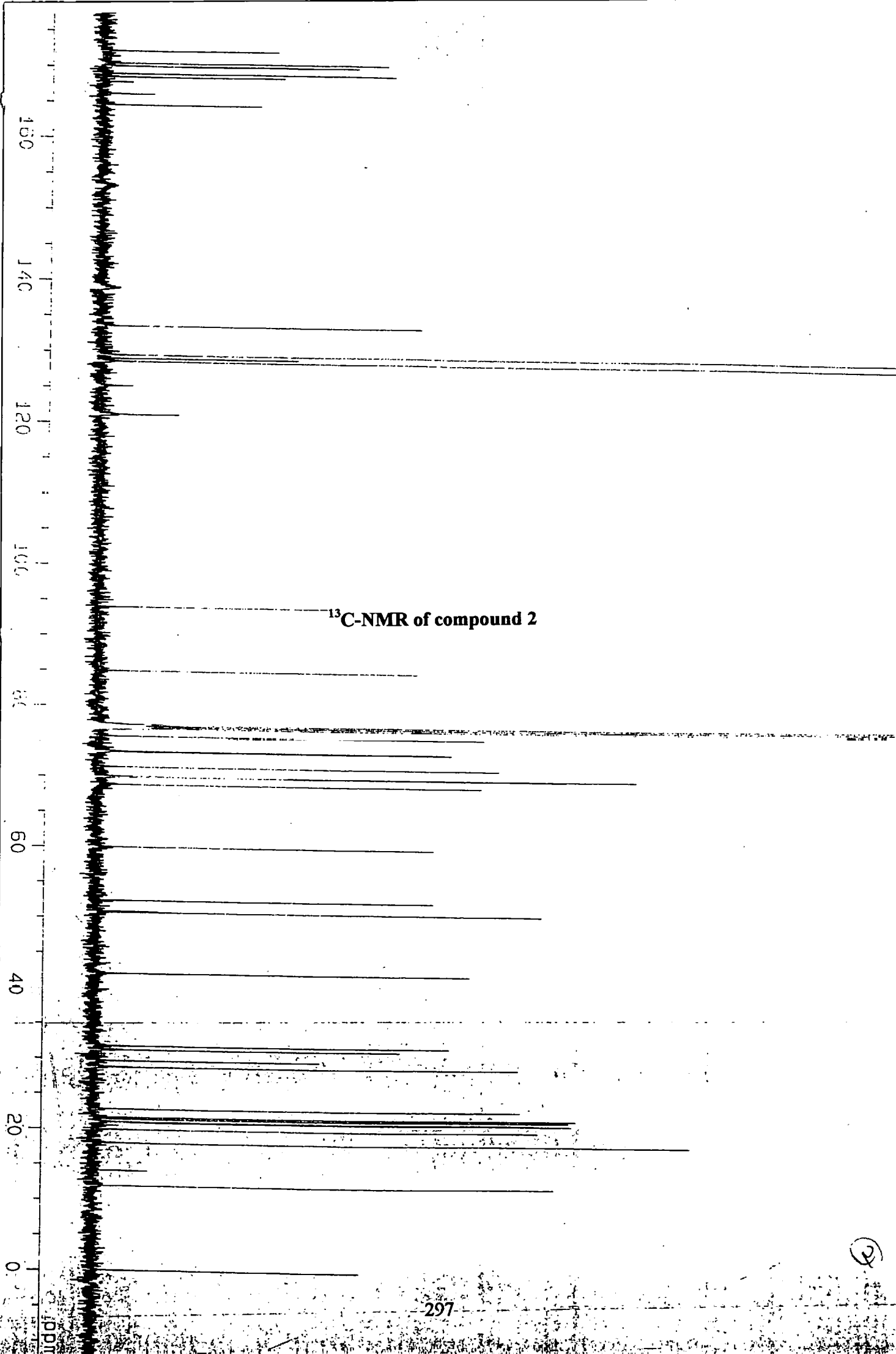
12.1963

1.1912
6.0660

12.4257

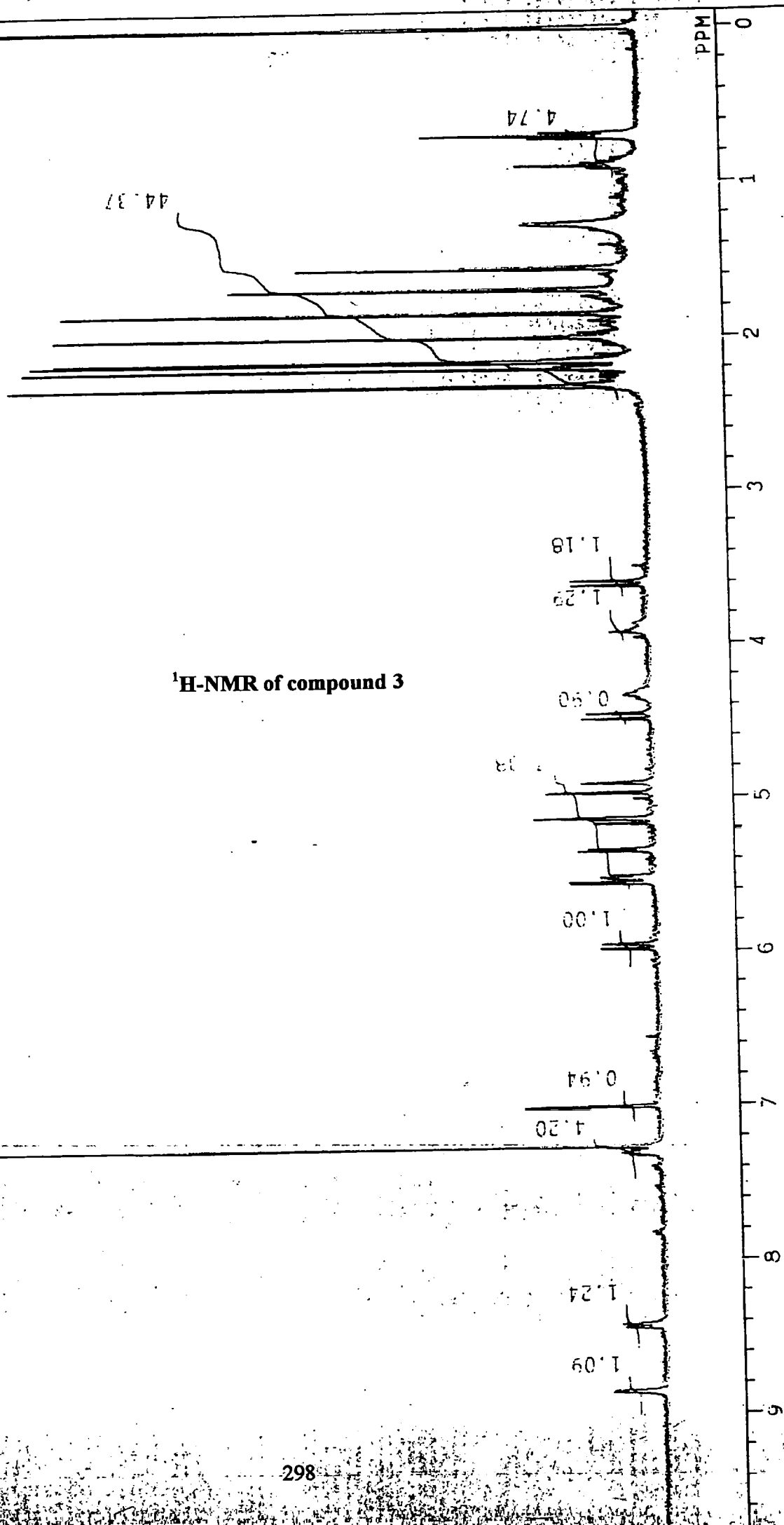
1.0000

1.1901



FOBNJG 1H
POINT
32762
4500.5 Hz
SCANS
16
SOLVENT CDCL3

¹H-NMR of compound 3



POINT 655.36
FREQ 30120.5 Hz
SCANS 1000
SLVNT CDCL3

^{13}C -NMR of compound 3

349
329
318
PPM

20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
PPM

299

3

Compound (4)

13.32

1.27

2.97

2.74

1.43

3.23

¹H-NMR of compound 4

1.00

1.00

0.47

1.00

300

4500.5 Hz

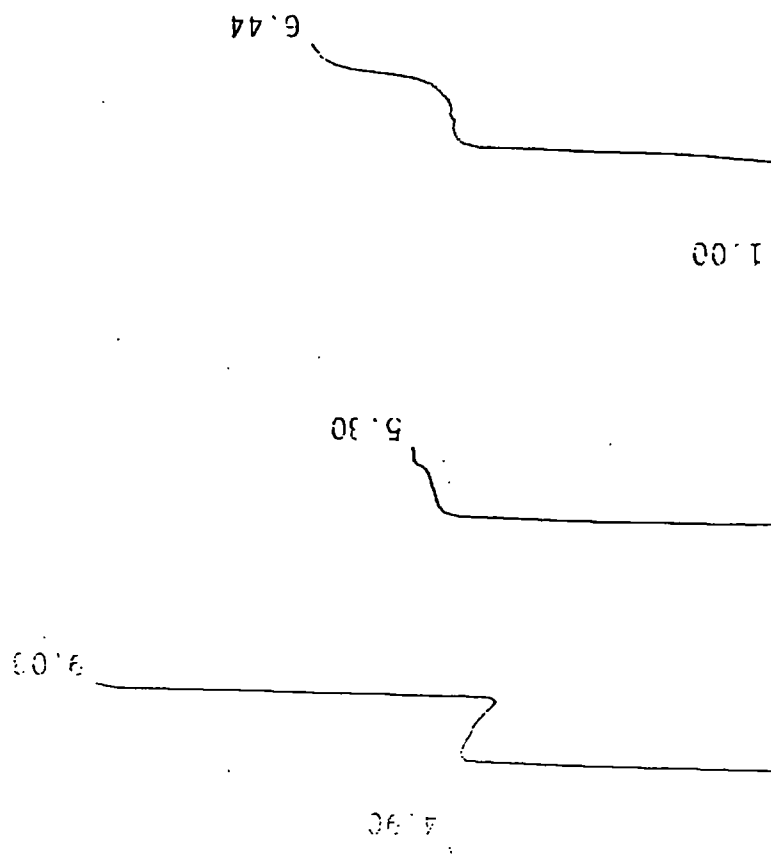
SCANS

RELVNT CDCL3

^{13}C -NMR of compound 4

77.349
77.029
76.709
PPM

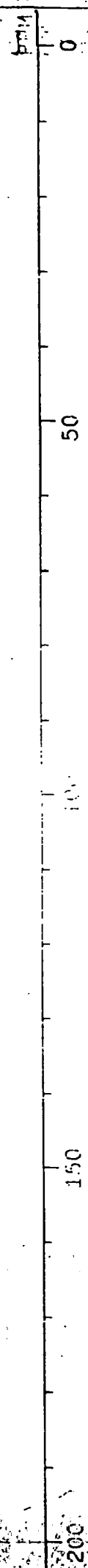
NAME: 1H-3000
NUC: 1H
POINT: 32708
FREQ: 500.0 Hz
SCANS: 16
SOLVENT: CDCL3



¹H-NMR of compound 5

DF-ILE HE45003
OBNUC 13C
POINT 65536
FREQ 23923.4 Hz
SCANS 500
SOLVENT CDCL3
EXMOD SGCOM
PD 1.300 sec
DE 1.00 Hz

¹³C-NMR of compound 5



370.3
500.0 Hz
CDCL₃

¹H-NMR of compound 6

2.94

1.00

2.00

1.00



¹³C-NMR of compound 6

